Bivalent Affective Modes in Central and Medial Amygdala

by

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Dedication

This dissertation is dedicated to my family and my Michigan family.

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"There is an art, it says, or rather, a knack to flying. The knack lies in learning how to throw yourself at the ground and miss… All it requires is simply the ability to throw yourself forward with all your weight, and the willingness not to mind that it's going to hurt."

-Douglas Adams, Hitchhikers Guide to the Galaxy

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Table of Contents

List of Figures

Abstract

How does the brain flexibly control motivation to obtain and avoid rewards, particularly when faced with multiple rewards? Affective mode hypotheses have proposed that striatal-like regions of the brain can adaptably flip between incentive and aversive motivation depending upon brain state, physiological inputs, and prior experience. This dissertation will present novel findings to demonstrate that reward choice can be incentively focused on a sugar or cocaine target, or even a shock-delivering object when paired with central amygdala (CeA) channelrhodopsin (ChR2) optogenetic stimulation, via recruitment of incentive-related circuitry. Further, CeA ChR2 stimulation was shown to be able to flip valence to enhancing aversive motivation when these same animals underwent paired Pavlovian fear learning. Next, I will highlight a role of the medial amygdala (MeA) in generating incentive motivation for one of two competing sucrose rewards and to a smaller extent, motivation to self-stimulate short bouts of MeA ChR2 laser-illuminations. Finally, I demonstrate that MeA can narrowly focus pursuit for an intravenous cocaine or remifentanil reward. Together these results are consistent with an extended amygdala macrosystem view in which CeA and MeA are functionally operating as striatal-like and can flexibly guide powerful incentive and aversive motivation.

CHAPTER I. Introduction

Motivation, whether conscious or unconscious, is a key driver of behavior throughout the animal kingdom (K. Berridge & Winkielman, 2003; L W Swanson, 2000). Over the course of evolution our brains have become excellent at detecting rewards and associated cues (e.g. food or a potential mate) in our environment and in turn generating motivation to pursue and obtain that reward (K C Berridge & Robinson, 2003; Kent C Berridge & Robinson, 2016; T. E. Robinson & Berridge, 1993).

The neuroscience of motivation has been largely evolutionarily conserved across species, ranging from vertebrates to invertebrates (including drosophila, rodents, horses, elephants, primates, etc.) (Anderson & Adolphs, 2014; Anderson, 2016; Kent C Berridge, 2004; Broca, 1878, 1978; Lee & Wu, 2020; McDonald, 1998). This has been eloquently demonstrated using anatomical and behavioral methods that involve tagging developing neurons along the developing neural tube and following the structure and function of these tagged neurons over the course of development (George F. Alheid et al., 1994; Bupesh, Legaz, Abellán, & Medina, 2011; Olsson, Björklund, & Campbell, 1998). While evolution has expanded upon the complexity of these structures in certain organisms, many of the embryonic origins may be traced across species and often traced to shared functions (Herrick, 1926; Johnston, 1923).

These behavioral neuroscience and neuroanatomical studies have further proved that mesocorticolimbic dopamine is released during salient events and used to imbue cues with incentive salience through midbrain dopamine release in limbic regions such as the nucleus accumbens (NAc), lateral hypothalamus (LH), amygdala, ventral pallidum (VP), parabrachial nucleus (PBN), dorsal striatum, orbitofrontal cortex (OFC), and insular cortex (Castro, Cole, & Berridge, 2015; Olney, Warlow, Naffziger, & Berridge, 2018; Richard, Castro, Difeliceantonio, Robinson, & Berridge, 2013; Warlow & Berridge, 2021; Warlow, Baumgartner, et al., 2020). Human literature has also shown that individuals with behavioral addictions or severe substance use disorder show heightened dopamine reactivity in these regions when presented with a reward-related cue (Boileau et al., 2016; Denomme & Shane, 2020; Garofalo & di Pellegrino, 2015; Gola et al., 2017; Joyner, Kim, & Gearhardt, 2017; Limbrick-Oldfield et al., 2017; O'Sullivan et al., 2011; Romer, Su Kang, Nikolova, Gearhardt, & Hariri, 2019; Wetherill et al., 2014).

While this mesocorticolimbic dopaminergic system is necessary for survival, it can easily become maladaptive in situations where access to rewards (such as calorically dense foods, drugs and alcohol) are present in abundance. Unfortunately this motivational system can become hypersensitive in a subset of individuals, or in research laboratories by activating mesolimbic regions, causing excessive desire and craving ('wanting') each time they come across a reward cue in their world (Kent C Berridge & Robinson, 2016; Corbit & Janak, 2007; Garbusow et al., 2019; Holmes, Marchand, & Coutureau, 2010; LeBlanc, Ostlund, & Maidment, 2012; Susana Peciña & Berridge, 2013; Susana Peciña, Schulkin, & Berridge, 2006; T. E. Robinson & Berridge, 1993; Warlow, Baumgartner, et al., 2020). A large goal in addiction neuroscience is to identify which structures and corresponding neuronal subtypes contribute to excessive desire to

take drugs, despite simultaneous desire (conscious wanting) to discontinue use. Accomplishing these preclinical goals will provide new treatment avenues for clinicians providing care for individuals with severe substance abuse or psychiatric disorders (Olney, Warlow, Naffziger, & Berridge, 2018). Applying these findings to theories of addiction, it suggests that addiction may not being driven by an individual's desire for pleasure ('liking') but rather a hyperactive recruitment of mesocorticolimbic circuitry in the brain whether occurring naturally in the human brain or artificially in animal models (Claus, Ewing, Filbey, Sabbineni, & Hutchison, 2011; Mahler & Berridge, 2009, 2012; M. J. F. Robinson, Warlow, & Berridge, 2014; Warlow, Robinson, & Berridge, 2017; Warlow, Baumgartner, et al., 2020).

A central theme that psychologists and affective neuroscientists have grappled with lies within the theoretical foundation of emotion (Damasio and Carvalho, 2013). How can the brain make sense of competing desires, for instance choosing between seeking food and seeking a mate? Or even choosing to leave the safety of the shelter to seek out food or a mate? One hypothesis that accounts for remedying the surplus of information and desires constantly bombarding the brain is the idea of affective valence operating via different modes rather than affective modules (Kent C Berridge, 2019; Tye, 2018). When this hypothesis is viewed with special appreciation of the hierarchical organization of motivated behavior in the brain, it accounts for the wide array of flexibility that the brain can show at any given moment depending upon previously learned experiences, situational factors, and current physiological states (de Olmos & Heimer, 1999; Lennart Heimer, Van Hoesen, Trimble, & Zahm, 2007; L W Swanson, 2000; Larry W Swanson, 2005). This dissertation will examine how optogenetic manipulations to central and medial nuclei of the amygdala can similarly promote or suppress motivated behavior in a flexible and situation-dependent manner.

The Extended Amygdala

A macrosystem view for understanding neuroanatomical hierarchical organization of the upper forebrain (telencephalon) described by Swanson and colleagues consists of viewing all structures within the entire forebrain telencephalon as either cortical, striatal or pallidal in level (L W Swanson & Petrovich, 1998; L W Swanson, 2000; Larry W Swanson, 2005). The cortex sends glutamatergic projections to striatal structures (embryologically arising from lateral ganglionic eminence), these striatal structures contain predominately GABAergic neurons, which in turn project to GABAergic pallidal structures (arising from medial ganglionic eminence(de Olmos & Heimer, 1999; Olsson, Björklund, & Campbell, 1998). From pallidal structures, information is sent to the thalamus for reentry to the cortex and to brainstem regions regulating the movement of motivated behavior (G F Alheid & Heimer, 1988; Lennart Heimer & Van Hoesen, 2006; L W Swanson, 2000; Larry W Swanson, 2005). While there is still considerable debate about whether this renaming of the lateral and medial ganglionic eminence to striatum and pallidum appropriately captures the embryonic origins and cytoarchitectural differences, evidence from behavioral neuroscience labs have supported this separation by identifying a special role to the striatal level structures as brain sites where local pharmacological microinjections or optogenetic manipulations can generate especially intense motivations (Baumgartner, Cole, Olney, & Berridge, 2020; Baumgartner, Schulkin, & Berridge, 2021; DiFeliceantonio & Berridge, 2012; Hong, Kim, & Anderson, 2014; Jennings et al., 2013; Miller, Marcotulli, Shen, & Zweifel, 2019; J. Nordman & Li, 2020).

While the concept of the extended amygdala gained momentum through carefully conducted anatomical studies trying to identify the sublentricular substantia innominata, early

evidence can be identified using neurodevelopmental studies comparatively across species (G F Alheid & Heimer, 1988; George F Alheid, 2003; De Olmos & Ingram, 1972; de Olmos & Heimer, 1999; L Heimer, Harlan, Alheid, Garcia, & de Olmos, 1997). Johnston implicated a continuum of neurons that shared a close relationship between the centromedial amygdaloid nuclei and the bed nucleus of the stria terminalis, and implicated possible overlap between reptiles indicated by Hendricks and in mammals such as rodents, rabbits, and primates (Herrick, 1926; Johnston, 1923).

Alheid and Heimer further elucidated the substantia innominata by dividing into three discrete but interwoven systems in the basal forebrain: striatopallidal system, extended amygdala, and corticopetal cell complex (G F Alheid & Heimer, 1988; L Heimer et al., 1997). The term extended amygdala was used to describe this continuum of neurons initially identified by Johnston, at the time typically referred to as the sublenticular substantia innominata, and reframed as operating within a corticostriatopallidal framework similar to the basal ganglia. In this view, the allocortex projects to the lateral portions of the central amygdaloid nucleus containing medium spiny neurons which in turn project to both the corticopetal cell complex for reentry into allocortex and to the medial portions of the central amygdaloid group which is viewed as acting as the pallidum. From the medial portions of the central amygdaloid group, projections are next sent to the hypothalamus, thalamus, brainstem and spinal cord. Within this proposed macrosystem view of the extended amygdala, it is the central amygdaloid nucleus, CeA, which is primarily thought to be capable of greatly influencing motivated behavior and the medial amygdaloid nucleus that is predominantly receiving associative projections that primarily project to the hypothalamus.

Importantly, the proposed theory above falls short in appreciating the contributions of medial amygdaloid nucleus (MeA) as being a driver in motivated behavior (R. K. Hu et al., 2021; Miller et al., 2019; J. Nordman & Li, 2020; J. Nordman, Ma, & Li, 2020; Smith & Torregrossa, 2021). While MeA has dense projections to the hypothalamus, it also projects directly to brainstem motor neurons that direct and help orchestrate a variety of motivated behaviors (DiBenedictis, Helfand, Baum, & Cherry, 2014; Dong, Petrovich, & Swanson, 2001; R. K. Hu et al., 2021; Newman, 1999; Petrovich, Canteras, & Swanson, 2001; Raam & Hong, 2021).

Importantly, Swanson's proposed macrosystem view indicates MeA as an important striatal-like region of its own right that can drive motivation flexibly for rewards. Viewing the extended amygdala from Swanson's proposed three-tier system view, the cortical nuclei include the basal, lateral, and piriform regions. Structures within the striatum include both the central and medial nuclei, with pallidal regions being viewed as the bed nucleus of the stria terminalis (G F Alheid & Heimer, 1988; ALHEID, 2006; George F. Alheid et al., 1994; George F Alheid, 2003; de Olmos & Heimer, 1999; L W Swanson & Petrovich, 1998; L W Swanson, 2000; Larry W Swanson, 2005). While both the central and medial nuclei fill the position of striatum, the extended amygdala can be further divided into two distinct systems: lateral extended amygdala and medial extended amygdala (Dong et al., 2001; Petrovich et al., 2001; L W Swanson, 2000; Larry W Swanson, 2005; Zahm, 2006). The lateral extended amygdala system contains the central amygdala (CeA) as a striatal-level structure, receiving cortical-type glutamate afferents from basolateral amygdala (BLA), and sending efferents to the lateral bed nucleus of the stria terminalis (lBNST), a pallidal-level structure. The lateral extended amygdala is traditionally the focus of neuroscience studies of motivated behaviors, involving food or drug rewards, fear, etc

(D. Funk, Li, & Lê, 2006; J. Kim, Zhang, Muralidhar, LeBlanc, & Tonegawa, 2017; Koob & Le Moal, 2008; Koob, 2013; Torruella-Suárez et al., 2020; Venniro et al., 2017; Warlow & Berridge, 2021; Zorrilla, Logrip, & Koob, 2014). In contrast, the medial extended amygdala system contains the medial amygdala (MeA) as the striatal-level structure, receiving glutamate cortical afferents from basomedial amygdala (BMA) and sending GABA efferents to mBNST, a pallidal level structure. The medial extended amygdala traditionally has been relegated to olfactory sensation functions, such as detecting sexual pheromones, and to reproductive function (Adekunbi et al., 2018; Bergan, Ben-Shaul, & Dulac, 2014; Fergani, McCarthy, Leon, & Navarro, 2019; Ferguson, Aldag, Insel, & Young, 2001; Frankiensztajn, Gur-Pollack, & Wagner, 2018; Hiura, Kelly, & Ophir, 2018; Kikusui et al., 2018; Y. Li et al., 2017; Newman, 1999; Yao, Bergan, Lanjuin, & Dulac, 2017). Functionally seated as the third relay in the olfactory processing circuit, MeA receives dense chemosensory input from olfactory and vomeronasal organ that allows for the manipulation of social behaviors appropriate upon detection of odor cues in the environment (Bergan et al., 2014; Canteras, Simerly, & Swanson, 1995; Kikusui et al., 2018; Y. Li et al., 2017; Newman, 1999; Takahashi, Hubbard, Lee, Dar, & Sipes, 2007; Westberry & Meredith, 2017).

Flexibility of Striatal-Structures

As previously mentioned, striatal-level structures are optimally placed to take higher-order cortical input, as well as homeostatic and physiological state updates, to generate intense motivated behavior. Imagine encountering the scent of your favorite home-cooked meal, the scent itself may make your mouth water and increase your motivation to seek out food. Now imagine that it has been a full day since you have eaten and you suddenly smell that favorite

dish, you would likely experience a much stronger motivational pull to obtain food. In this moment you may even choose to forgo your preferred food option and instead eat something that's easily accessible. This is due to these striatal structures that receive information about physiological hunger signals, cognitive desires, etc. when making the decision to seek a food source. In addition to receiving information about physiological state, striatal regions receive dopamine from the midbrain, which has been suggested to mediate the attribution of incentive salience to reward cues and invigorate appetitive behavior (Kent C Berridge & Robinson, 2016; T. E. Robinson & Berridge, 1993).

It is with this constant update of information that the brain may shift into different affective modes to guide behavior. Perhaps the most eloquent initial experiments that captured this flexibility in motivation occurred in the nucleus accumbens (NAc), a widely-accepted striatal structure within the ventral striatopallidal system. In these studies, rats receiving microinfusions of DNQX into NAc both increased positive motivation to consume food and fearful motivation depending upon the external environment (Baumgartner et al., 2020; Faure, Reynolds, Richard, & Berridge, 2008; Faure, Richard, & Berridge, 2010; Richard, Plawecki, & Berridge, 2013). When rats were tested in a familiar environment, the DNQX microinfusions caused excessive eating, whereas motivation to eat was instead replaced with fearful treading when rats were tested in a bright and loud, noisy environment.

The second chapter will identify affective modes within the central amygdala by investigating pairing photostimulation of CeA with one of two competing positive rewards, a controllable shock, an uncontrollable shock, and a neutral object. In doing so we'll reveal situations in which 'wanting' becomes focused on a particular target whether positive, negative, or neutral in the environment when optically paired with CeA stimulation dependent upon

situational factors. Importantly, depending upon the environment, this focused motivation can lead to excessive incentive salience for rewarding sugar and cocaine and even dangerous desire for a painful, shock-delivering object. However, when the situation flips so that the voluntary, controllable shock is replaced with an uncontrollable predicted footshock in a Pavlovian fear-conditioning situation, photostimulating these same neurons in CeA replaces increased incentive motivation with increased fear motivation as measured by freezing to an auditory CS+ and avoidance of a CS+ odor. Histological analysis of c-fos reveals that the same reward structures are activated following CeA channelrhodopsin (ChR2)-paired sucrose, cocaine, and surprisingly the controllable shock. Histological analysis of encounters with the uncontrollable shock reveals very different patterns of activation from the rewards and controllable shock, with more recruitment of regions involved in fear expression (J. J. Kim & Jung, 2006; J. E. LeDoux, Iwata, Cicchetti, & Reis, 1988; Maren, 2001; Penzo et al., 2015). These findings demonstrate that central amygdala activation is capable of inducing attraction towards pain and cues associated with pain when access to the painful stimulus is under full control of the rat. However, when the painful shock becomes uncontrollable, as in the conditioned fear paradigm, the affective mode flips from being incentive motivation to aversive motivation.

The third chapter of this dissertation identifies a role of the neglected medial extended amygdala in reward behaviors outside of purely social interactions and highlights a powerful affective modules for pleasure within the medial nucleus of amygdala (MeA). In doing so, we reveal that optogenetically stimulating MeA neurons increases appetitive motivation for a rewarding sucrose pellet, a controllable shock-delivering object in a small percentage of animals, and to a lesser extent a neutral object, similar to paired CeA stimulations. In a conditioned fear

paradigm, MeA ChR2 did not significantly alter freezing to an auditory CS+ but did significantly enhance avoidance of the CS+ odor in a conditioned place avoidance task.

The fourth chapter of this dissertation will further explore the positive affective mode within MeA while given the option to self-administer intravenous cocaine or remifentanil. Here MeA ChR2 is able to induce a preference for the paired remifentanil reward and to a lesser extent paired cocaine. While most rats developed a significant preference for their MeA ChR2 paired drug choice, it was not quite as strong as the preference seen with CeA ChR2 drug choice and failed to escalate across days.

Together, these results compliment the anatomical model proposed by Swanson and was functionally demonstrated in behaving rats. We were able to take two equal rewards and arbitrarily make one more sought out and 'wanted' by recruitment optogenetic stimulation of neurons in CeA and MeA. These data provide support for extended amygdala striatal-like regions being powerful drivers in motivation and able to flexibly shift between affective modes.

CHAPTER II. The Central Amygdala Recruits Mesocorticolimbic Circuitry for Pursuit of Reward or Pain.

Introduction

The amygdala and related mesocorticolimbic circuitry help assign motivational significance to both reward-related and threat-related stimuli (Baxter & Murray, 2002; DiFeliceantonio & Berridge, 2012; Holland & Gallagher, 2003; J. LeDoux, 2007; Mahler & Berridge, 2012; Weiskrantz, 1956). In clinical disorders, maladaptive attractions can become intense and narrowly focused on inappropriate targets, as in addictions and self-harming (Bechara et al., 2019; Edmondson, Brennan, & House, 2016). Here we explore amygdala-triggered mechanisms that induce maladaptive attractions, operationally defined as attractions that are excessively intense (e.g., more than double the attraction than the same target supports in ordinary individuals), simultaneously narrowly focused (e.g., attraction pulled nearly entirely to one target, among otherwise equally attractive targets), and which carry adverse consequences (e.g., pain).

Intense and narrowly focused attractions have been induced in laboratory rats by pairing of optogenetic channelrhodopsin (ChR2) stimulation of neurons in central nucleus of amygdala (CeA) with sensory rewards, intensifying appetitive motivation (e.g., effort breakpoints) and

narrowing pursuit to the paired reward in choice tasks (M. J. F. Robinson et al., 2014; Tom, Ahuja, Maniates, Freeland, & Robinson, 2019; Warlow et al., 2017). We report here that CeA ChR2 pairings can further narrow attraction at experimenter's whim to either a natural sucrose reward or cocaine drug reward when both rewards are available, thus arbitrarily making a rat into either a 'sucrose addict' that ignores alternative intravenous cocaine, or conversely a 'cocaine addict' that ignores sucrose. Further, CeA ChR2 pairing can create maladaptive attraction to a noxious stimulus, such as an electrified shock rod, which normally elicits avoidance and fear-related defensive reactions (De Boer & Koolhaas, 2003; Treit, Pinel, & Fibiger, 1981). The value of motivation produced by CeA ChR2 pairings can also switch to negative valence in a traditional Pavlovian fear learning context, oppositely increasing conditioned defensive reactions to cues for the uncontrollable footshock. Finally, CeA ChR2 valence can further switch to relatively neutral when laser is delivered by itself or paired with innocuous stimuli. Thus, CeA ChR2 control of mesocorticolimbic circuitry can create either maladaptive attractions, exaggerated fear reactions, or become relatively neutral by interacting with situational factors.

Methods

Animals

Male and female rats (N = 55; female Sprague-Dawley = 37; male Sprague-Dawley N = 6; female Long Evans Hooded N = 12) that weighed between 250 and 300 g before surgery were housed in rooms maintained at ~21 °C on a reverse 12-h light/dark cycle; males and

females were housed in separate rooms and always tested separately in clean chambers. Rats had ad libitum access to water and food (Purina Lab Chow) in their home cages throughout the experiment. Prior to experiments, rats were handled at least 5 days for 10 min each day. The University of Michigan's Committee on the Use and Care of Animals approved all procedures.

Optogenetic virus infusion and optic fiber implant

Each rat was anesthetized with 5% isoflurane anesthesia, and received atropine (0.04 mg/kg; Henry Schein) prior to surgery, and was maintained at 2–3% isoflurane throughout the surgery. A 0.75 µl volume of optogenetic virus containing a gene for channelrhodopsin with human synapsin promoter (AAV5-hSyn-ChR2-eYFP, n = 39) was microinjected bilaterally into the CeA (A/P from Bregma in mm: −2.4, M/L: 4, D/V: −7.6 with mouth bar set to −3.3; 0.1 µl/min for 10 min microinjection). Sites were slightly staggered across individuals to be distributed throughout CeA. Control group rats received an optically inactive virus at similar bilateral sites in CeA (eYFP; AAV5-hSyn-eYFP, N = 16). In the same surgery, all rats were implanted with bilateral optic fibers aimed 0.3 mm above the intended CeA site, and fibers were secured to skull screws with a dental acrylic headcap. Rats were subcutaneously injected with cefazolin sodium (60 mg/kg, Henry Schein) to prevent infection, and carprofen (5 mg/kg, Henry Schein) as a post-surgical analgesic.

Intrajugular catheter implantation

In a separate surgery 2 weeks later, rats intended for cocaine self-administration tests (N = 19; females = 14; males = 5) were anesthetized again as above and were implanted with an intravenous catheter in the jugular vein10. Silastic intrajugular catheters (0.28 mm internal diameter) were threaded into the right jugular vein, then passed subcutaneously along the dorsal

neck and secured to an anchor exiting from the dorsal mid-scapular region. Rats were allowed 10 days recovery before beginning any behavioral tests. Intrajugular catheters were flushed daily with 0.2 ml sterile isotonic saline solution containing 5 mg/ml gentamicin sulfate (Sparhawk, KS) for 2 weeks, and by sterile saline alone thereafter, to prevent infections or clogs. Catheter patency was tested once before behavioral testing, and again after the end of all tests, by intravenous injection of 0.2 ml methohexital sodium to induce ataxia (20 mg/ml in sterile water, JHP, MI). Rats that became ataxic within 10 s were considered to have a patent catheter and included in analyses.

Sucrose vs. cocaine instrumental choice

Choice training and tests (sucrose vs. cocaine) were carried out in modified MedAssociates chambers (30.5 × 24.1 × 21.0 cm) with clear Plexiglas floors, which contained two instrumental nosepoke portholes on a side wall. Nose-poking into either porthole was detected by infrared beams and recorded by MedPC software. For some rats, these portholes were retractable, so that they were usually flush with the wall and occluded, but the outer rim could enter through the wall to protrude in the chamber, while the inner center did not protrude, to present an active porthole able to earn cocaine or sucrose. After the presentation, the porthole was retracted again to disappear, while the other porthole entered the chamber or a time-out ensued. This was meant to mimic the presentation and disappearance of retractable levers in previous studies that used levers as instrumental manipulanda (Lenoir, Augier, Vouillac, & Ahmed, 2013; Warlow et al., 2017). For other rats, two standard portholes remained fixed in place throughout the entire session, to mimic other previous studies in which nosepokes earned intravenous cocaine (Deroche-Gamonet, Belin, & Piazza, 2004; Warlow et al., 2017). This difference ensured that our results were not limited to either procedure. An infusion pump

outside the chamber delivered intravenous liquid cocaine delivery via tygon tubing. Nosepokes into the cocaine porthole always earned a 50 μL intravenous infusion of 0.3 mg cocaine (weight of the salt; donated by NIDA, Lot# 13722-21C) per kg weight of the rat, dissolved in isotonic saline, infused over a 2.8-s period. Sucrose pellets were delivered into a recessed dish in the chamber wall between the portholes via food hopper. Since choice between cocaine and sucrose may partly depend on the palatability of the sucrose pellet (Lenoir, Serre, Cantin, & Ahmed, 2007; Nair, Gray, & Ghitza, 2006), we used two different sucrose pellets for different rats. For some rats, nosepokes on their sucrose porthole earned a 45 mg nearly pure-sucrose pellet (N = 8; LabTabsTM, TestDiet, Richmond, IN), and for other rats, it earned an even more preferred 45 mg sucrose candy pellet that also contained milk fat and casein as well as sucrose (N = 8; AIN-76A, TestDiet, Richmond, IN). A video camera placed below the transparent floor recorded all behavior for subsequent off-line analysis of consummatory behaviors, such as chewing on the portholes10.

Rats were first trained in 60-min daily sessions with a single active porthole and single daily reward for 6–10 days until each rat attained a criterion of earning a cumulative total of 50 sucrose rewards and 50 cocaine rewards. Training days alternated between earning either sucrose exclusively and cocaine exclusively, each through nosepokes on its own instrumental porthole, until criterion was reached for both outcomes. Subsequent tests used simultaneous 2-choice presentations of both portholes, allowing either or both rewards to be earned. Some rats were randomly chosen and designated to be permanently ‘Laser+Cocaine rats’. Others were permanently designated to be ‘Laser+Sucrose rats’. All rats had met criterion already for earning both rewards. For each rat, bilateral blue laser illumination (473 nm, 10 mW, 25 Hz, 8-s duration)

for optogenetic CeA excitation was always paired with earning their laser-designated reward, beginning immediately with nosepoke registration and continuing as they received the cocaine infusion or consumed the sucrose pellet (Laser + Sucrose: N = 5 ChR2 and N = 2 control eYFP; Laser + Cocaine: N = 6 ChR2 and N = 3 control eYFP). Earning their alternative reward was never accompanied by CeA laser.

On given training days 1–8, a rat could earn only cocaine, or else only sucrose, by nosepokes into its particular porthole designated for that reward14. The other porthole produced no outcome during that day (if porthole was fixed) or was not present (if retractable). The next day, nosepokes into the second porthole earned the alternative reward, while the first porthole was inactive or not present. This pattern continued until the end of training (when rats earned a total of 50 cocaine and 50 sucrose rewards). Some rats began training with cocaine reward on the first day, while other rats began with their sucrose reward. All rats also received auditory Pavlovian CS cues via wall speaker to mark successful earning of each porthole's reward outcome (tone or white noise; 8 s); assignment of tone/white noise auditory cues to sucrose or cocaine was always consistent for a given rat, but balanced across rats. Amount of days to reach criterion of earning 50 rewards was equal whether laser was paired with sucrose or cocaine (ChR2, N = 11 vs. eYFP, N = 5; two-way ANOVA: $F_{1, 12} = 1.49$, $p = 0.25$).

At the beginning of a 2-choice test session, one randomly selected porthole was first presented or made operative alone until its reward was earned. Then after a 20-s time-out the alternative porthole was presented until the rat earned its other reward. This sequence was

repeated again, so the rat earned two cocaine rewards and two sucrose rewards (forced-sampling) immediately prior to making a choice. This was done to be sure that each rat re-experienced both rewards, ensuring equal priming, and to avoid danger of the rat becoming trapped into simply choosing the first reward encountered.

Subsequently during each of the 2-h session, both portholes were always presented simultaneously, allowing a 2-choice decision, so the rat could choose which reward it preferred to earn. These simultaneous presentations were repeated for up to 10 times each session, allowing 10 consecutive choices to be made. Once a choice was made and earned by a nosepoke, its outcome was delivered (sucrose pellet or 0.3 mg/kg cocaine infusion as in training) accompanied by its associated auditory cue. After each choice was made, a 10-min time-out was imposed before the two portholes again became operative or presented (Lenoir et al., 2013). Each rat also received bilateral CeA laser with each of its individually designated outcome, either sucrose or cocaine, but never with the alternative outcome (473 nm, 10 mW, 25 Hz, 8 s bin illumination). This entire choice procedure was repeated daily for another 3 days.

CeA laser self-stimulation tests

To assess whether CeA ChR2 excitation was an independent incentive or reinforcer by itself, rats were tested for CeA laser self-stimulation. In an active spout-touch task, rats could earn laser illumination on a FR1 basis by actively touching a designated empty-metal spout. Rats were placed in MedAssociates operant chambers in which two novel and empty sipper spouts protruded ~5 inches apart from the back wall of the chamber. Each touch upon a spout closed a circuit between spout and metal grid floor, and was recorded. One spout (designated as ‘laser spout’; spout assignment counterbalanced across rats) delivered a 1 or 8 s CeA laser stimulation

each time it was touched (25 or 40 Hz, 10 mW, 1-s duration: N = 11, or 8-s duration: N = 7). The 1-s pulse duration was used because it has supported robust optogenetic self-stimulation in previous studies18. The 8-s pulse duration was assessed in separate tests because it replicated the laser parameters that controlled motivation for laser-paired cocaine or sucrose in 2-choice task above. The second spout never produced laser, and simply served as a control to assess baseline exploratory touches on a similar object. Each session lasted 30 min. Rats were considered to be laser self-stimulators if the made at least twice as many laser spout touches than inactive spout touches, and made >50 touches.

Laser-paired aversive shock rod

In a separate experiment with different rats (ChR2: N = 25; eYFP: N = 11), we paired voluntary encounters with an aversive "shock rod" with CeA ChR2 stimulation in order to compare effects of CeA stimulation with a negative-valenced outcome. In this situation, all encounters with shock are under the rat's instrumental control, and it can conversely choose to avoid shocks. In that sense, instrumental shock pursuit would be similar to instrumental pursuit of sucrose or cocaine rewards, but with an outcome of opposite affective valence (aversive electric shock). The shock rod (1.5 × 1.5 × 9 cm core, wrapped with electrified wire along its full length) protruded 9 cm into one side of a Plexiglas chamber containing 2-cm depth of corn cob bedding scattered on the floor (chamber: 38-cm width × 38-cm length × 48-cm height; bedding: Bed'O'Cobs, Andersons Inc., Maumee). The bedding was present to allow defensive burying behavior, which is normally elicited from rats that encounter the shock rod (12, 13, 54). Touching the rod delivered a 0.2–0.5 mA (depending on <0.25- to >1-s duration of contact; measured using in-house ammeter), which continued as long as contact was maintained (duration <1/5th s). Touching the rod was never forced, but each rat touched at least once while

exploring the chamber. Any subsequent touches were purely voluntary, as the rod occupied under 2% of the floor area of the chamber. A video camera recorded behavior throughout each session for subsequent off-line analysis.

On the initial shock-rod day, rats were attached to bilateral optic fiber delivery cables, placed into the middle of the chamber and allowed to freely move around and explore the chamber in a 20-min session. Upon first contact with the shock rod, usually with forepaw or sometimes with snout, a mild shock (0.20 mA) was delivered to the skin. The rat typically withdrew contact reflexively and terminated the shock within 50–100 ms. Laser illumination began when any part of a rat approached within 2 cm of the rod (473 nm, 10 mW, 40 Hz (5-ms ON, 20-ms OFF, triggered via MATLAB program), and continued until the rat withdrew further than 2 cm away from the rod. Approaches within 2 cm were 95% of the time accompanied by shock, so laser activation bracketed the shock before and after for a second or so, typically with a 3–8 s total duration. Sometimes the rat touched the rod again before withdrawing, which accounted for the longest laser illuminations. Our intention in this was to paste CeA ChR2 excitation on the entire perceptual encounter with the shock-delivering rod, rather than on the brief (typically < 0.1–0.25 s) shock alone, similar to laser duration in sucrose and cocaine encounters in the previous study. A subset of rats (ChR2: N = 8, eYFP: N = 6) also heard an auditory Pavlovian CS+ whenever within 2 cm of shock rod and laser was illuminated, with the same duration as laser (tone or white noise, counterbalanced between rats). This was intended to provide an additional sensory CS+ label for encounters with the shock rod. The alternative CS− sound (either white noise or tone) was presented later that day in a separate session the same number of times as the CS-, in a similar chamber with bedding but in a different room and with no rod present. This roughly equated the number of presentations of CS+ and CS− sounds. All

behavior was video-recorded for off-line scoring later. Identical shock rod and laser sessions were repeated on days 2 and 3. On the fourth day of training, a laser-extinction session was run, similar to previous days and with the rod still electrified, but no laser illumination was delivered. This laser-extinction test assessed whether CeA ChR2 established learned changes in behavior toward the rod that were enduring, or instead depended on actual CeA ChR2 excitation during the test.

Instrumental conditioned reinforcement test

The hypothesis that CeA ChR2 promotes motivation in part by attributing incentive salience to cues for the paired UCS target implies that Pavlovian CS+s for an attractive target become attractive themselves. Attraction to the shock rod provides a powerful test of this hypothesis, as it implies that an auditory CS+ label for shock might become paradoxically attractive to CeA ChR2 rats. We assessed the attractiveness of the auditory CS+ associated with shock in CeA ChR2 rats and control eYFP rats by asking if they would learn to perform an instrumental nosepoke response to earn presentations of either the auditory CS+ alone or the equally familiar CS− (ChR2: N = 8, eYFP: N = 6). This instrumental conditioned reinforcement test occurred on 2 separate days in a MedAssociates chamber. Rats were presented with two novel fixed portholes (these rats had never previously learned to nosepoke for any reward, so porthole nose-poking was an entirely new instrumental response for them). On the CS+ day (balanced order), a nosepoke into one designated porthole earned a 4-s presentation of the auditory CS+ that previously had been paired with shock-rod encounters (FR1; either tone or white noise for different rats; responses were considered ‘CS+ pokes’). Nosepokes into the other porthole produced nothing, and were recorded to assess baseline pokes due to general activity or exploration (‘CS+ Inactive poke’). On the other CS− day, a nosepoke into the active port now

produced a 4-s presentation of the CS− sound (white noise or tone; 'CS− pokes'), while the other port still delivered nothing ('CS− Inactive poke'). The number of nosepokes in each porthole was recorded. Each daily session lasted 30 min, and order of CS+ and CS− days was counterbalanced.

Motivated rod approach? Overcoming sudden barrier

To further test whether shock-rod approach by CeA ChR2 rats was flexibly motivated, in the sense of being willing to overcome a novel barrier suddenly placed in their way in order to get to the rod, CeA ChR2 rats and control eYFP rats with 3 days of previous shock-rod experience were given a barrier test. The sessions began with 5 min of free access to the rod as in days 1–3, with both shock and laser conditions activated (473 nm, 10 mW, 40 Hz laser). After 5 min, an opaque barrier (37-cm length × 13-cm width × 13-cm height, cardboard box wrapped in a blue pad) was inserted in the middle of the chamber between the rat and the shock rod, gently nudging the rat if needed to block its access to the rod. The barrier occluded the rat's view of the shock rod, and physically prevented approach unless the rat actively climbed 13 cm over the barrier to reach the rod. Behavior was video-recorded for another 10 min, for subsequent off-line analysis of climbs, latency to reach the rod, and touches (Noldus Observer XT 12).

Pavlovian fear conditioning

To further examine CeA ChR2 in a standard defensive fear conditioning paradigm, in which CeA has been implicated in threat learning, naive rats (ChR2: N = 8; eYFP: N = 5) were trained for 3 consecutive days to learn a Pavlovian association between an auditory CS+ and an unavoidable UCS 0.5-s footshock (0.75 mA) (Ciocchi et al., 2010). During training on the first day, after a 3-min habituation period, three CS+/UCS pairings were presented and separated by

60 s fixed inter-trial intervals. The auditory CS+ was a 10-s tone (80 db at 5 kHz), and accompanied by bilateral CeA laser illumination (473 nm, 10 s, 40 Hz, 10 mW) during training. The UCS was 0.5-s footshock scrambled across the grid floor (500 ms, 0.75 mA) that followed immediately after termination of CS+ (did not overlap). In addition, another contextual olfactory CS+ cue was present during shock conditioning trials (either almond or lemon essence, counterbalanced; applied by task wipes (KimTech Science)). The alternative contextual CS− odor was separately presented in the homecage in sessions equal in number and duration. After the three pairings of CS+ on day 1, an additional two pairings were presented on day 2, and a final one pairing was presented on day 3. Pavlovian freezing as a conditioned response (CR) to the auditory CS+ was tested on day 4 in a distinctly different chamber, which had a plexiglass floor (not metal grid) with a different odor (Versaclean) and house light. After a 1-min baseline period, a series of 10 CS+ tones were presented, each separated by 60 s. During five of these presentations (order randomized), bilateral CeA laser illumination was delivered for 10 s concurrently with the CS+ tone (10 s, 40 Hz, 10 mW). The other five CS+ presentations occurred alone, without CeA laser. On a subsequent day, rats were tested for contextual CS+ odor avoidance in a place preference/avoidance chamber for 30 min: one chamber was scented with the footshock-associated contextual CS+ odor, and the other chamber with CS− odor (scented wipes placed underneath the chambers; sides of CS+ odor assignment counterbalanced between rats). The two chambers were also distinguished by different visual patterns on the walls to aid discrimination (stripes or polka dots). Time spent in each compartment was video-recorded and subsequently scored offline using Noldus Observer Software.

Wooden block/food intake: CeA stimulation and general motivation to eat

We explored the effect of CeA laser stimulation on voluntary food consumption in a 60 min free-intake test. Rats (N = 8 ChR2, N = 4 eYFP) were tested in a familiar homecage environment with bedding on the floor, and had continuous access to pre-weighed quantities of food (Purina Lab Chow; ~20 g) and water. Behavior was video-recorded, and at the end of each session, remaining chow weight and water volume was recorded again to calculate the amount consumed. A pre-weighed wooden block (~18 g) was also available to allow non-ingestive chewing, and was re-weighed at the end. The first day was considered a familiarization procedure to encourage a reliable baseline. Intake tests were repeated the next 2 consecutive days to obtain baseline vs. laser measures. Laser stimulation was administered only on 1 day, occurring either on day 2 or 3 (ABA or AAB design, counterbalanced across rats) in 15-s ON–9-s OFF alternations (40 Hz; 20-ms ON, 5-ms OFF; 10 mW), and the other 2 days served as baseline comparisons. Cumulative time spent eating, drinking, or chewing during laser and nonlaser sessions was scored offline using Noldus Observer software.

Histological analyses of virus expression and Fos plumes

Beginning 75 min prior to euthanasia and perfusion, CeA laser stimulations with parameters similar to those that had produced incentive effects were given to rats in sucrose–cocaine choice (N = 7) and in shock-rod encounter (N = 16) groups. Laser stimulation was either accompanied by cocaine-sucrose choice (ChR2: N = 4) or shock-rod situations (ChR2: N = 12 and eYFP: N = 4) to re-activate CeA-induced systems and behavioral incentive effects simultaneously. Another control group of unoperated and naive rats, never before exposed to any experimental situation, were taken directly from homecage for euthanasia and perfusion, to allow comparison to measure normal baseline levels of Fos expression (in the absence of cocaine,

sucrose, shock-rod or related stimuli, and without surgical penetration, gliosis, virus infection or light/heat insults to neural tissue; N = 4).

After 75 min from the onset of any above condition, rats were deeply anesthetized with an overdose of sodium pentobarbital (150–200 mg/kg) and transcardially perfused using ice-cold PBS followed by ice-cold 4% PFA. Brains were post-fixed for 24 h in 4% PFA, cryoprotected in 30% sucrose PBS, and coronally sectioned at 40 µm using a cryostat (Leica). For immunohistochemistry, sections were first blocked in 5% normal donkey serum/2% triton-X PBS solution for 30 min, incubated for 24 h in a polyclonal rabbit anti-cfos IgG primary antibody (1:1000, Santa Cruz Biotechnology), followed by 2 h in AlexaFluor anti-rabbit IgG secondary antibody (3:1000, Life Technologies). All sections were mounted, air-dried, and cover-slipped with anti-fade Pro-long gold (Invitrogen). For each CeA placement, images surrounding the fiber optic tip were taken at ×10 magnification, using a Leica microscope and Oasis surveyor software. Immunoreactivity for Fos protein and virus expression were visualized using filters with excitation bands 515–545 and 490–510, respectively. Number of Fos+ (or eYFP+) cells were counted in 15 successive blocks (50 × 50 µm) along eight radial arms that emanated from the fiber optic tip. Counting continued along each arm until at least two consecutive boxes were zero, at which point marked the radius of that arm. Fos elevation was calculated as % change from either of two baselines: (1) Illuminated inactive-virus control levels: equivalent block locations from CeA of eYFP control rats that received laser illumination prior to perfusion similarly to ChR2 rats, or (2) Normal tissue baseline: counts of Fos from CeA in unoperated control brains of normal rats. Fos elevations in ChR2 blocks were denoted in increments of

>200% elevation or higher >300% elevation above the respective two mean baselines (Warlow et al., 2017).

Fos quantification in distributed brain circuitry

Oasis Surveyor software was used to capture tiled images of whole brain coronal section at ×10 magnification pre-determined by Paxinos and Watson brain atlas (Paxinos & Watson, 2007) and using a filter with 515–545 excitation band to visualize Fos expression. Whole brain images were used to count Fos protein at multiple sites in orbitofrontal cortex, insula, basolateral amygdala, nucleus accumbens core and shell, ventral pallidum, ventral tegmentum, periacqueductal gray, and lateral hypothalamus. For each brain region, three sites each in anterior, posterior, and middle regions were separately counted under treatment-blind conditions. For each site (at each anterior-posterior site), three 100 × 100 × 40 μm boxes were placed onto the coronal brain image in Adobe Photoshop software by those blind to experimental conditions. To ensure site placements were consistent between rats, placement of the three boxes for each subregion were guided by a template plotted on a brain atlas page corresponding to the structure.

Statistical analysis

Mixed ANOVAs were used to analyze within-group effects (e.g., laser pairings and on/off conditions) and between-group differences (e.g., CeA ChR2 vs. CeA eYFP groups). Significant ANOVAs were followed by parametric paired t-tests and independent t-tests to analyze specific post hoc comparisons of conditions (using either Bonferroni or Dunnett's two-sided tests). Data found to not have normal distributions were analyzed with nonparametric one-way ANOVAs followed by nonparametric paired t-tests. Each test used a confidence interval of

95% with a significance level of $p < 0.05$, two-tailed. Finally, Cohen's d was used to calculate effect sizes among pairwise comparisons.

Results

CeA ChR2 virus and Fos protein expression

Laser stimulation of ChR2-infected CeA neurons produced local zones of excitation in CeA reflected in local Fos plumes of 0.15–0.2 mm radius around optic fiber tips. Plumes contained >200–300% elevations in Fos, compared with baseline levels in eYFP or unoperated controls (Fig. 2.1a, b and Supplementary Fig. 1). Fos plume diameters were used to determine the size of 0.4 mm placement symbols for functional maps in figures (Fig. 2.1c, d). CeA ChR2 laser pairings also activated distant brain circuitry as described below.

Sucrose vs. cocaine two-choice task

We first assessed the effect of pairing CeA ChR2 stimulations with earning either sucrose or cocaine in an instrumental nose-poke task, when rats were given choices between the two rewards (Lenoir et al., 2013, 2007) (Fig. 2.2a). Control rats with optically inactive virus in CeA ('eYFP') chose about equally between sucrose and cocaine regardless of which was paired with laser (Fig. 2.2b, c). By contrast, CeA ChR2 rats with amygdala laser stimulation paired with sucrose continually pursued and consumed only sucrose, ignoring cocaine. Conversely, different CeA ChR2 rats with laser stimulation paired with cocaine, exclusively pursued cocaine, while ignoring sucrose (both 87 + 4% preference by day 4, or a 10:2 ratio, compared with eYFP 1:1 ratio of 49 + 13%; Fig. 2.2d).

CeA ChR2 rats were also >30 times faster to initiate nosepokes into their laser-paired porthole than into their nonlaser porthole, once each was available on single-choice trials (within 3 ± 0.3 s; median: 2), regardless of whether their laser-paired porthole earned sucrose or cocaine

(97 ± 12 s; median: 40) (N = 11, Wilcoxon signed-ranks test: Z = 2.8, p = 0.005). After earning a reward, CeA ChR2 rats continued to perseverate in making repeated additional nosepokes specifically in their laser-paired porthole during the ensuing 8-s time-out period (5 ± 1 total perseverative pokes), despite perseverative responses earning nothing (Supplementary Fig. 2.2c).

CeA ChR2 pairing also increased consummatory actions targeted toward associated metal cues. CeA ChR2 rats nibbled and bit their laser-paired porthole twice as much as they nibbled/bit the nonlaser porthole regardless of whether the laser-paired reward was cocaine or sucrose (Supplementary Fig. 2.2d). Increases in consummatory actions directed toward Pavlovian cues for reward is a sign of heightened incentive salience, which can make cues become perceived as more orally attractive and consumable (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Tomie, Grimes, & Pohorecky, 2008; Warlow et al., 2017).

CeA ChR2 pairing creates attraction to noxious shock rod

In a different situation with a noxious shock rod, separate groups of CeA ChR2 and eYFP rats received pairings of CeA laser each time they voluntarily approached within 2 cm of the electrified rod (laser 40 Hz; 10 mW; bin duration 1–8 s, depending on how long the rat remained within 2-cm proximity of shock rod; shock rod = 1 cm in diameter and 9-cm long, wrapped with electrified wire that delivered 0.2–0.5-mA shock, depending on <0.25- to >1-s duration of contact) (Fig. 2.3a).; also see Supplementary Fig. 2.2a, b).

Control eYFP rats quickly learned to avoid the shock rod after touching it once or twice, and remained as far as possible from the rod for the remainder of the 20-min session. eYFP rats

often emitted an active species-specific antipredator reaction of defensive treading–burying directed toward the shock rod (Fig. 2.3a) (De Boer & Koolhaas, 2003; Treit et al., 1981). This often resulted in a small mound of cob bedding gradually being built around the rod during the 20-min session (Fig. 2.3b). Eighty-eight percent of eYFP rats emitted defensive treading/burying bouts longer than 10-s duration, and 75% of eYFP rats did so even on the first day (Supplementary Fig. 2.3a).

By contrast, CeA ChR2 rats approached and touched the rod five times on average the first day, receiving five shocks, touched and received seven shocks on the second day, and touched and received eight shocks on the third day (Fig. 2.3c; Supplementary Fig. 2.3b). Upon each shock, CeA ChR2 rats reacted immediately with reflexive startle and withdrawal reactions, just as eYFP control rats did, suggesting electric shock retained aversive impact during laser stimulation (Fig. 2.3a). But after receiving a shock, CeA ChR2 rats typically returned within seconds or minutes to the rod, continually hovering closely over it, and soon received another shock to paw or mouth (Fig. 2.3c). Only one CeA ChR2 rat emitted any defensive treading–burying bout of longer than 10-s duration on the 3 days, and that rat did so only once (36% of CeA ChR2 rats never showed any antipredator behavior at all on any day; Fig. 2.3b).

Consummatory chewing and sniffs of rod

CeA ChR2 rats additionally emitted occasional consummatory actions of chewing, nibbling, or biting on the metal shock rod during bouts of continuous rod sniffing. At least 66% (14/21) of CeA ChR2 rats nibbled or chewed the rod at least once on the first day, 71% (15/21) on the second day, and 66% (14/21) on the third day, on which they spent an average cumulative duration of 81 ± 24 s of oral nibbling or chewing on the shock rod (e.g., 5% of a 20-min session;

Fig. 2.3c, Supplementary Fig. 2.3d). Consequently, CeA ChR2 rats often incurred shocks directly on their mouth, tongue or teeth, or on their nose while sniffing too closely (0.2 mA intensity within 0.5 s of chewing, reaching 0.4–0.5 mA within 2–3 s according to ammeter readings). A few chewing bouts reached up to 10–20-s duration (typically composed of several 2–4 s continual chewing bouts separated by brief <1 s withdraws/pauses). By contrast, no eYFP control rat ever nibbled or bit the rod on any day (Fig. 2.3d and Supplementary Fig. 2.3c).

Oral consummatory actions were likely not a simple motor effect of CeA ChR2 activation. The same rats failed to increase chewing of an inedible wooden block paired with ChR2 stimulation in separate tests, and there was no individual correlation between duration of shock-rod chewing and wooden block chewing (Pearson correlation, $r = 0.049$, $p = 0.92$). Similarly, CeA ChR2 laser stimulation did not cause greater touches of the wooden block (Supplementary Fig. 2.4b). In a separate test, laser pairings also failed to induce attraction to a dummy "no-shock rod" that was nearly identical to shock rod but was unelectrified. CeA ChR2 rats never chewed on the dummy rod, and touched the dummy rod no more often than eYFP controls (Supplementary Fig. 2.4a).

CeA ChR2 attraction to shock rod, once established, appeared robust across a range of optogenetic laser frequency and intensity parameters. Subsequent tests with paired laser frequencies of either 10, 25, or 40 Hz at 10 mW intensity, or constant illumination of laser at 1 mW intensity (constant low illumination is thought to facilitate endogenous firing patterns, rather than impose an artificial firing frequency (Kravitz & Kreitzer, 2011), all produced similar levels of shock-rod attraction and chewing in CeA ChR2 rats as the original 40 Hz 10 mW laser stimulation (Supplementary Fig. 5), suggesting CeA ChR2 attraction does not depend on any particular single laser parameter.

To assess if shock-rod attraction required concomitant CeA stimulation, rats were re-exposed to the electrified shock rod on a separate 'laser extinction' day, during which CeA laser illumination was no longer administered. All CeA ChR2 rats initially approached and touched the rod at least once, but ceased chewing the rod almost entirely after receiving the first shock, and 7/8 ChR2 rats reduced rod approaches and touches to less than half their number on the previous day when laser had last been delivered (Fig. 2.3c). Further, ChR2 rats also began to emit short bouts of defensive treading/burying (averaging ~5 s) toward the rod for the first time (Fig. 2.3b). We conclude CeA ChR2 rats remain able to recognize the noxious qualities of shock rod, and their full level of attraction to the shock rod is not simply due to a permanent learned re-evaluation but also depends in part on simultaneous rod-paired CeA ChR2 stimulations during the session.

Motivated rod attraction overcomes obstacle

We next assessed if CeA ChR2 rats were motivated to overcome an obstacle to reach the shock rod, when it was not immediately perceived. A large opaque obstacle block was interposed between the rat and the shock rod early in a session. The block completely filled the width of the chamber, prevented easy viewing of the rod, and required the rat to climb over it in order to reach the rod (Fig. 2.4a). All ChR2 rats actively climbed over the block (5/5) to touch the shock rod, upon which they were returned to the other side of the barrier. CeA ChR2 rats persisted in repeatedly climbing over the block 5 ± 1 times and received 3.36 ± 1 shocks per 15 min session, compared with eYFP rats that made 0 or 1 crosses, and did not receive shocks. CeA ChR2 rats also still typically chewed on the shock rod once they reached it (>5-s bouts), whereas eYFP rats never chewed (Fig. 2.4a).

CeA ChR2 rats 'want' shock-associated cues

Given that incentive salience typically makes Pavlovian reward cues become 'wanted' themselves (Kent C Berridge & Robinson, 2016), we assessed whether shock-rod cues gained their own incentive value by asking whether CeA ChR2 rats that had been attracted to shock rod would 'want' to hear an auditory Pavlovian cue associated with shocks (distinctive auditory tone or white noise presented during rod encounters and CeA laser stimulations; counterbalanced across rats). In an instrumental conditioned reinforcement test, conducted in novel chambers with shock-rod absent (Fig. 2.4b), rats were given the opportunity to nosepoke to earn 4-s presentations of either the shock-associated auditory cue (CS+) on 1 day, or an alternative auditory cue that had been heard in their homecage (CS−) on a separate day. During both days, nosepokes into another porthole earned nothing, serving as a control for general exploration ('Inactive'). CeA ChR2 rats reliably worked to hear their shock-associated CS+ sound repeatedly, making >300% greater nosepokes for CS+ presentation than for their CS− presentation (homecage sound) (Fig. 2.4b). By contrast, eYFP rats worked at much lower levels, and did not significantly discriminate between CS+ and CS− sounds (1.2:1 ratio).

Shock-rod attraction activates mesocorticolimbic circuity

We next assessed what brain circuitry was activated in CeA ChR2 rats that were attracted to shock rod, or in ChR2 rats that exclusively pursued either laser-paired sucrose or laser-paired cocaine, by measuring Fos protein expression in mesocorticolimbic brain structures after a final test session.

CeA ChR2 rats pursuing laser-paired sucrose or cocaine showed a pattern of Fos elevation in several limbic structures: ventral tegmental area (>800% activation vs. baseline;

Supplementary Table 1), rostromedial NAc shell (>700% activation), and posterior insula (>500% activation). CeA ChR2 rats also showed Fos elevation in dorsolateral neostriatum (>500% activation), and conversely showed an opposite >200% reduction below baseline tissue levels in ventrolateral periaqueductal gray area (PAG) and basolateral amygdala.

CeA ChR2 shock-rod attraction induced a similar pattern of mesocorticolimbic activation, with a >400% Fos elevation above eYFP control levels in the midbrain ventral tegmental area (VTA), particularly in the caudal half of VTA (Fig. 2.5), and >200% elevation in nearby substantia nigra pars compacta (SNc), consistent with activation of dopamine projection neurons (Steinberg et al., 2020). CeA ChR2 shock-rod rats also showed >180% elevation in the rostral medial shell of nucleus accumbens (NAc). NAc Fos was not elevated in the caudal half of medial shell, nor in either rostral or caudal NAc core. In neostriatum, CeA ChR2 Fos was elevated by 200%, particularly in the dorsolateral quadrant of neostriatum. In the basal forebrain, >200% elevation was found in the perifornical region of lateral hypothalamus. In limbic cortex regions, CeA ChR2 Fos was elevated ~175% in medial orbitofrontal cortex (mOFC) and >250% in posterior insula over eYFP levels.

Conversely, shock-rod avoidance among eYFP rats showing defensive behavior was associated with a separate pattern of Fos elevation over CeA ChR2 levels. Specifically, eYFP rats had >400% Fos elevation in the ventrolateral periacqueductal gray (PAG), >240% elevation in the basolateral nucleus of amygdala, and >125% Fos elevation in the bed nucleus stria terminalis (BNST) (Supplementary Table 1).

<u>CeA stimulation potentiates Pavlovian fear responses</u>

CeA has well-known roles in fear learning and defensive motivation, as well as in reward motivation (Averbeck & Costa, 2017; Beyeler et al., 2018; Haubensak et al., 2010), but these are usually tested in different situations. Many fear-related amygdala studies use a Pavlovian conditioned freezing situation, in which an auditory CS+ sound predicts a footshock unconditioned stimulus (UCS) that is uncontrollable, inescapable, and of relatively high magnitude. We therefore assessed if such situations could cause the motivational effects of paired CeA ChR2 stimulation to flip valence from positive to negative in naive CeA ChR2 and eYFP rats, including some that had previous shock-rod experience. During Pavlovian fear training, a 10-s tone (CS+) predicted an unavoidable footshock UCS (0.75 mA, 500 ms) (Fig. 2.6a). CeA laser illumination began with CS+ onset and continued through UCS footshock (40 Hz, 10 mW). A distinctive olfactory contextual cue (CS+Context scent) was also paired with the Pavlovian fear conditioning chamber.

During test sessions on another day, CS+ tones were presented alone (without footshock UCS), and elicited freezing as Pavlovian conditioned responses (CRs) in both ChR2 and eYFP rats (Fig. 2.6b; ChR2 (N = 8) and eYFP (N = 5) freezing baselines; two-sided unpaired t-test: $t11 = 3.12$, $p = 0.01$). CRs were assessed as percent increase in freezing over pre-CS+ baseline levels by the same rat (i.e., normalized to avoid pre-existing differences between groups). Some CS+ presentations were accompanied by CeA laser as during training, whereas other test presentations of CS+ occurred without laser, to assess whether CeA ChR2 laser stimulation altered the expression of freezing CRs elicited by CS+s. Results showed an interaction between CeA laser activation and ChR2/eYFP groups in duration of freezing CRs elicited by CS+s (Fig. 2.6b), with ChR2 rats emitting longer duration freezing CRs when CS+s were accompanied by

CeA laser than when CS+s occurred without laser. This pattern indicates that concurrent CeA ChR2 stimulation magnified the expression of freezing CRs.

Independently, avoidance of the olfactory CS+Context cue associated with Pavlovian footshock UCS was examined in a separate test of place avoidance (Fig. 2.6c). CeA ChR2 rats displayed avoidance of the place scented with CS+Context odor, whereas eYFP rats did not (eYFP failure to show CS+Context avoidance is consistent with other reports of context-specificity for odor-footshock conditioning, as very different chambers were used here for Pavlovian fear training and odor-place tests (Otto, Cousens, & Rajewski, 1997). Avoidance of CS+Context by CeA ChR2 rats indicates CeA ChR2 laser stimulation enhanced the acquisition of Pavlovian contextual fear learning during training, as laser was never administered during olfactory avoidance tests and could not have magnified avoidance CR expression. Thus overall, our results suggest CeA ChR2 stimulation in a traditional Pavlovian fear conditioning paradigm can increase both the acquisition and expression of Pavlovian defensive CRs.

Failure of CeA laser self-stimulation

Does CeA ChR2 stimulation have valence or motivational value of its own? We assessed CeA ChR2 valence (alone without shock, sucrose, or cocaine) in laser self-stimulation tests for all rats above, using a spout-touch task. Touching one empty-metal spout earned brief laser illuminations (either 8-s or 1-s duration), whereas touching a different spout earned nothing (and merely served as a baseline measure of exploration) (Fig. 3.7a) (Kravitz & Kreitzer, 2011). We found that only a minority of CeA ChR2 rats in the sucrose/cocaine group (3 of 10) and the shock-rod group (4 of 19) met criteria for robust laser self-stimulation (defined as greater than twice as many touches on laser spout as on nonlaser spout, and >50 touches/self-stimulations per

session). These seven self-stimulating ChR2 rats earned ~100–300 laser stimulations per session. The remaining 22 ChR2 rats made only 10–40 touches on both spouts, similar to eYFP rats. Thus, overall, the CeA ChR2 rats as a combined group failed to self-stimulate CeA laser significantly (Fig. 3.7c, d).

This general lack of self-stimulation was notable since laser had powerfully controlled pursuit of shock rod, sucrose or cocaine, even in the same ChR2 rats that failed to self-stimulate. CeA ChR2 rats that did self-stimulate from the shock-rod group failed to show greater shock-rod attraction than non-self-stimulators ($N = 4$ self-stimulators, $N = 14$ non-self-stimulators; two-sided unpaired t-test: $t_{16} = 0.15$, $p = 0.88$), and ChR2 self-stimulators from the sucrose/cocaine groups showed no stronger pursuit of their laser-paired sucrose/cocaine reward than other CeA ChR2 rats that failed to self-stimulate ($N = 7$, laser-spout preference × laser-paired sucrose/cocaine preference, Pearson correlation: $r = -0.17$, $p = 0.69$). ChR2 self-stimulators from the Pavlovian fear conditioning group showed laser potentiation of defensive freezing CRs as strong as non-self-stimulators. However, the three strongest laser self-stimulators from shock-rod group chewed more on the shock rod than non-self-stimulators (140 ± 68 s cumulative chewing duration for self-stimulators vs. 31 ± 16 cumulative s for rats that failed to self-stimulate; $t_{16} = 2.4$, $p = 0.029$, 95% CI: −205, −13, $d = 1.03$).

Discussion

Pairing CeA ChR2 stimulation with sucrose, cocaine, or shock encounters produced strong motivation that switched between positive valence and negative valence, depending on situation. CeA ChR2 stimulation paired with earning sucrose produced single-minded pursuit and consumption focused on sucrose while the rats ignored intravenous cocaine. CeA ChR2 pairing with cocaine for other rats produced pursuit and consumption focused solely on cocaine while they ignored sucrose. CeA ChR2 pairing with shock-rod encounters produced maladaptive attraction to repeatedly approach, touch, and even nibble the shock rod, despite consequently receiving multiple electric shocks.

Our shock-rod findings reveal that a stimulus with aversive properties can become an incentive target when paired with appropriate limbic activation, leading rats to subject themselves repeatedly to noxious shocks in an apparently compulsive fashion. The aversive shock from the rod was itself an important component of CeA ChR2-induced attraction, as a nearly identical laser-paired 'dummy rod' without shock failed to become attractive. Thus, CeA ChR2 induction of 'wanting what hurts' may provide the strongest proof of principle demonstration available so far that strong mesocorticolimbic 'wanting' can be induced in complete absence of 'liking'.

ChR2 expression via human synapsin (hSyn) promoter indiscriminately infects most CeA neurons, regardless of neurobiological type. Future studies could examine whether CeA neuronal sub-populations (e.g., SOM+, PKC+/−, CRF+, or D1 vs. D2 dopamine receptors, etc.), which have been suggested to play distinct roles in motivated behavior (Cai, Haubensak, Anthony, &

Anderson, 2014; Fadok et al., 2017; J. Kim et al., 2017; Torruella-Suárez et al., 2020; Venniro et al., 2020) make differential contributions to the CeA ChR2 effects on motivation reported here (Fadok, Markovic, Tovote, & Lüthi, 2018; J. Kim et al., 2017; Steinberg et al., 2020; Tye, 2018).

CeA ChR2 stimulations also recruited neurobiological activation among other structures within mesocorticolimbic circuitry to control pursuit of sucrose, cocaine and shock-rod targets. For example, within nucleus accumbens, maladaptive attractions recruited Fos elevation especially in the rostral half of medial shell, which contains a functional hedonic hotspot where opioid, endocannabinoid and related neurochemical signals enhance 'liking' reactions, and which is especially implicated in generating positively-valenced motivation even when unaccompanied by 'liking' (S Peciña & Berridge, 2000; Susana Peciña & Berridge, 2013; Richard & Berridge, 2011b). In limbic cortex, Fos was also elevated in an anteromedial subregion of orbitofrontal cortex and a posterior subregion of insula; both of those cortical subregions also contain hedonic hotspots (Castro & Berridge, 2017; Kringelbach, 2005). This suggests mesocorticolimbic structures traditionally associated with positive valence functions were recruited in order to mediate maladaptive attraction to the shock rod. By contrast, control eYFP rats that fearfully avoided the shock rod, and instead emitted defensive treading, recruited activation of different limbic structures, such as bed nucleus of stria terminalis (BNST), basolateral amygdala, and midbrain periaqueductal gray, which are implicated in anxiety, fear and pain (Deng, Xiao, & Wang, 2016; Goode, Ressler, Acca, Miles, & Maren, 2019; Marcinkiewcz et al., 2016).

Several observations suggest that mesolimbic incentive salience, or 'wanting', was a psychological contributor to CeA ChR2 attraction. One signature feature of incentive salience

attribution is to make Pavlovian cues attractive themselves. Such incentive cues become sought out when absent, and elicit approach when present (Flagel, Akil, & Robinson, 2009; Robbins, Watson, Gaskin, & Ennis, 1983). For example, here CeA ChR2 rats sought out an auditory cue associated with shock rod when absent, showing willingness to work in a new nose-poke task to hear the CS+ sound associated with shock-rod encounters. Similarly, CeA ChR2 rats that were initially unable to see the shock rod due to an occluding barrier, were willing to climb over the barrier to reach the rod.

Consistent with cue attraction, CeA ChR2 rats interacted with the shock rod in ways suggesting the rod had become an attractive cue for shock, rather than seeking out electric shocks per se. That is, 'wanting what hurts' may not be the same as 'wanting to be hurt'. For example, CeA ChR2 rats did not simply throw themselves upon the rod as though seeking shock, but instead continuously examined the rod with close sniffs, bringing face and paws close to the rod. CeA ChR2 rats even sometimes appeared to hold back their paw from the rod for a moment as though avoiding shock, yet eventually their fascination would bring paw or nose too close, and so receive a shock.

Another signature feature of incentive salience is that consummatory behaviors often become directed toward a CS+ cue, such as nibbling on a metal lever or porthole associated with reward (Bindra, 1978; Tomie et al., 2008). Here, CeA ChR2 rats typically nibbled or bit their shock rod, and others nibbled their laser-paired cocaine or sucrose portholes more than their nonlaser reward porthole (Tom et al., 2019; Warlow et al., 2017). Increased consummatory biting may be related to other reports of CeA ChR2 induction of biting in mice, such as by

activation of CeA projections to the brainstem parvocellular reticular formation (Han et al., 2017). However, biting here did not appear to simply be either a direct aggressive or motor effect, given that CeA ChR2 rats did not typically bite their laser-paired unelectrified dummy rod or wooden block.

A third feature of incentive salience is that 'wanting' intensity is often modulated by relevant physiological states and brain states (appetites, stress, intoxication, etc.) (Kent C Berridge, 2012; Olney et al., 2018; M. J. F. Robinson & Berridge, 2013; Zhang, Berridge, Tindell, Smith, & Aldridge, 2009). Here, CeA ChR2 attraction to the shock rod appeared to be enhanced and maintained by the current brain state. When laser was discontinued, rod attraction faded quickly and was replaced by avoidance and defensive treading. This suggests that, rather than persisting as a permanent learned attraction, simultaneous pairing of amygdala stimulations with shock-rod encounters are needed to continually re-boost the attractiveness of the rod and keep it 'wanted'.

CeA ChR2 induction of 'wanting' does not necessarily imply that it also enhances 'liking'. To the contrary, a previous study indicated that CeA ChR2 stimulation failed to enhance orofacial 'liking' reactions to sucrose taste, despite increasing 'wanting' to pursue sucrose9. Further, CeA ChR2 stimulation here potentiated negatively-valenced defensive freezing and avoidance responses in the Pavlovian fear conditioning paradigm. Fear CR potentiation is opposite to what would be expected if CeA ChR2 stimulation caused 'liking' of paired targets.

A related question is whether CeA ChR2 stimulation reduced the perceived unpleasantness of shock, given that CeA circuitry regulates analgesia and pain reactions (e.g., CeA SOM+ vs. PKC-delta-expressing neurons (Paretkar & Dimitrov, 2019; Wilson et al., 2019). Here, ChR2 rats that were attracted to the shock rod still reacted with brief flinching reactions to each shock. CeA ChR2 pairings also potentiated defensive conditioned freezing and avoidance responses in the Pavlovian fear conditioning situation, oppositely to what would be expected from analgesia. Still, it is possible that CeA ChR2 stimulation was accompanied by partial oral analgesia for the shock rod in a few individuals: CeA ChR2 rats tended to emit more biting on the shock rod if they also self-stimulated laser, consistent with a potential correlation between self-stimulation and partial analgesia. However, an alternative explanation is equally possible, namely, that CeA ChR2 pairing induced stronger incentive salience in those individuals, both promoting rod biting and enabling enough incentive attribution to the ordinarily-resistant neutral laser spout to motivate self-stimulation, without necessarily being accompanied by analgesia. We conclude that analgesia, while possibly a minor component contributing to prolonged rod biting, was not a primary mechanism of CeA ChR2 attraction.

Why did stimuli such as sucrose, cocaine, shock rod, or footshock all support induction of strong CeA ChR2 motivations, whereas other laser-paired stimuli (e.g., wooden block, dummy rod, or an empty-metal spout for laser self-stimulation) usually evoked weaker or no motivation? One potential explanation is that the eligible target stimuli were all motivationally potent even before laser pairing, in the sense that they could have served as affective unconditioned stimuli (UCSs) to establish motivated Pavlovian conditioned responses. Such affective UCSs could be expected to recruit activation in corresponding mesocorticolimbic

circuitry. It is possible that stimulation of CeA ChR2 neurons together with simultaneous UCS-activation of mesocorticolimbic circuitry combines synergistically to create stronger ChR2-induced motivation, which then becomes narrowly focused on the associated target. By contrast, relatively neutral stimuli, such as a self-stimulation spout, dummy rod or wood block, fail to trigger much mesocorticolimbic activity and consequently may remain weaker targets for CeA ChR2-paired motivation.

Our finding that CeA ChR2 pairings potentiated negatively-valenced defensive freezing and avoidance CRs in the traditional Pavlovian fear situation is consistent with many reports of CeA involvement in Pavlovian fear learning (Ciocchi et al., 2010; Kochli, Thompson, Fricke, Postle, & Quinn, 2015; Wolff et al., 2014), but contrasts with CeA ChR2 induction of single-minded appetitive pursuit of cocaine, sucrose, or shock rod here, and other demonstrations of CeA roles in appetitive motivation (Servonnet, Hernandez, El Hage, Rompré, & Samaha, 2020). CeA ChR2 motivations thus appeared to reverse here between positive and negative valence in different situations, sometimes even within the same individual rat: inducing positive incentive attraction to the shock rod, but amplifying negative fearful reactions to Pavlovian cues for footshock.

What determines switches in CeA valence? One possible explanation is that situational and target stimulus factors interact with CeA neuronal stimulation to determine the valence, as well as intensity, of motivational salience imparted to the paired target. Motivational salience is known to be able take two forms of opposite valence: positively-valenced incentive salience or negatively-valenced fearful salience (Kent C Berridge, 2012; Olney et al., 2018). Incentive

salience makes the attributed target more powerfully 'wanted' and attention-grabbing, able to elicit approach and trigger seeking and reward consumption. Fearful salience makes its target equally attention-grabbing, but as a potential threat percept that elicits defensive reactions, including the antipredator reaction of defensive treading–burying observed here (Reynolds & Berridge, 2001; Richard & Berridge, 2011a). In humans, mesolimbic fearful salience has also been suggested to contribute to human paranoia (Howes & Kapur, 2009; Olney et al., 2018).

Several features of the Pavlovian fear conditioning situation might have helped tilt the CeA ChR2 valence balance toward fearful salience, whereas the shock-rod situation remained biased toward incentive salience. For example, negative valence in Pavlovian fear conditioning might dominate in part because the noxious footshock was unavoidable and inescapable, and was not spatially localizable to a place within the chamber (Fanselow, 1980). By contrast, shocks from the rod were spatially localizable and under voluntary control, occurring only when the rat actively approached and touched the rod (De Boer & Koolhaas, 2003; Treit et al., 1981). Also, Pavlovian footshocks were physically more intense at 0.75 mA than the 0.2–0.5 mA shocks from the shock rod (however, shock-rod shocks were often received on mouth and tongue, which may somewhat offset the higher intensity of footshocks). Future studies will be needed to explore the roles of these or other factors in determining the CeA ChR2 valence.

Do maladaptive attractions described here have potential clinical implications? Important features of addictive motivation include maladaptive motivated pursuit that becomes focused narrowly on the addictive target (Ahmed, 2010; Heyman & Dunn, 2002), escalation of consumption, and persistence despite adverse consequences. Narrowly focused pursuit and

escalated consumption were seen here and in previous effort-breakpoint studies (M. J. F. Robinson et al., 2014; Tom et al., 2019; Warlow et al., 2017). Our results show that single-minded CeA ChR2 pursuit can be generated and focused narrowly on an incentive target of experimenter's choice, arbitrarily creating either a 'sucrose addict' that ignores cocaine, a 'cocaine addict' that ignores sucrose, or even maladaptive attraction to a painful shock rod.

In humans, conceivably, sufficient endogenous CeA activations paired with affective targets might eventually produce similar focused, addictive motivations in susceptible individuals. CeA-induced shock-rod attraction further indicates that an aversive stimulus can itself become a target of compulsive attraction, under certain conditions that recruit mesocorticolimbic incentive circuitry. Maladaptive attraction occurred here without need of hedonic reward, and in absence of any pre-existing habits, in contrast to some contemporary models that posit those features to be required for addictive compulsions (Deroche-Gamonet et al., 2004; Lesscher & Vanderschuren, 2012; Vanderschuren & Everitt, 2004).

Beyond addictions, CeA ChR2 induction of 'wanting for what hurts' may also suggest a potential alternative explanation for some cases of pursuit of pain or harm, such as self-cutting. Although traditional explanations of self-harm typically rely on coping strategies (Rasmussen, Hawton, Philpott-Morgan, & O'Connor, 2016; Sandy, 2013), our results suggest that, under some conditions, maladaptive 'wanting' can occur directly via incentive motivation processes recruited and focused on a pain-associated target.

Finally, our results showing CeA ChR2 reversal between positive attractions and negative fear potentiation underscores the potential motivational plasticity of CeA circuitry, suggesting the possibility of multiple affective modes for CeA neuronal function. That is, the motivational evaluation of a stimulus may not reside either inherently in the encountered physical stimulus or simply in a momentary CeA activation, but rather in a brain's individualized representation of their combination, flexibly gated by situational factors.

Figures

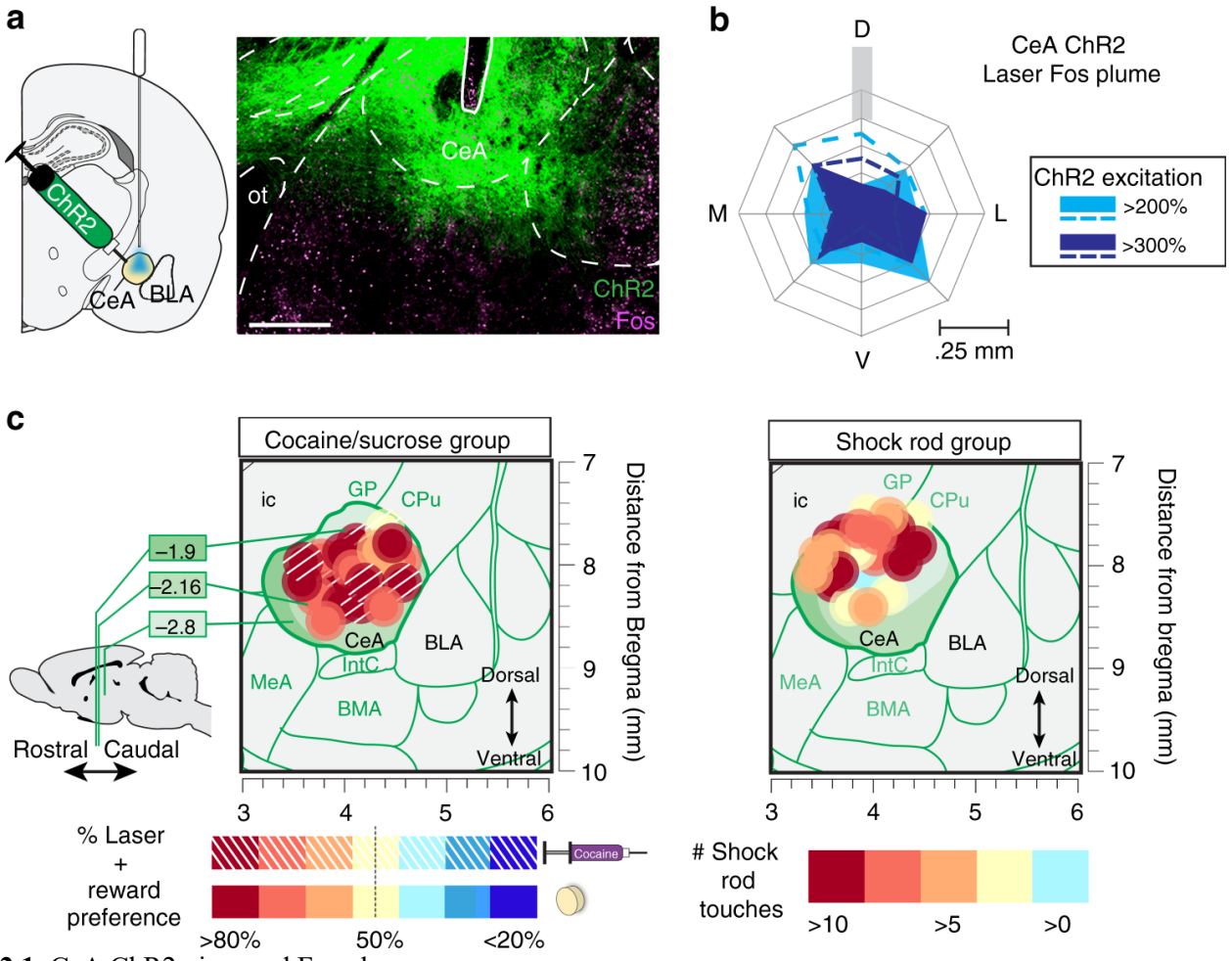
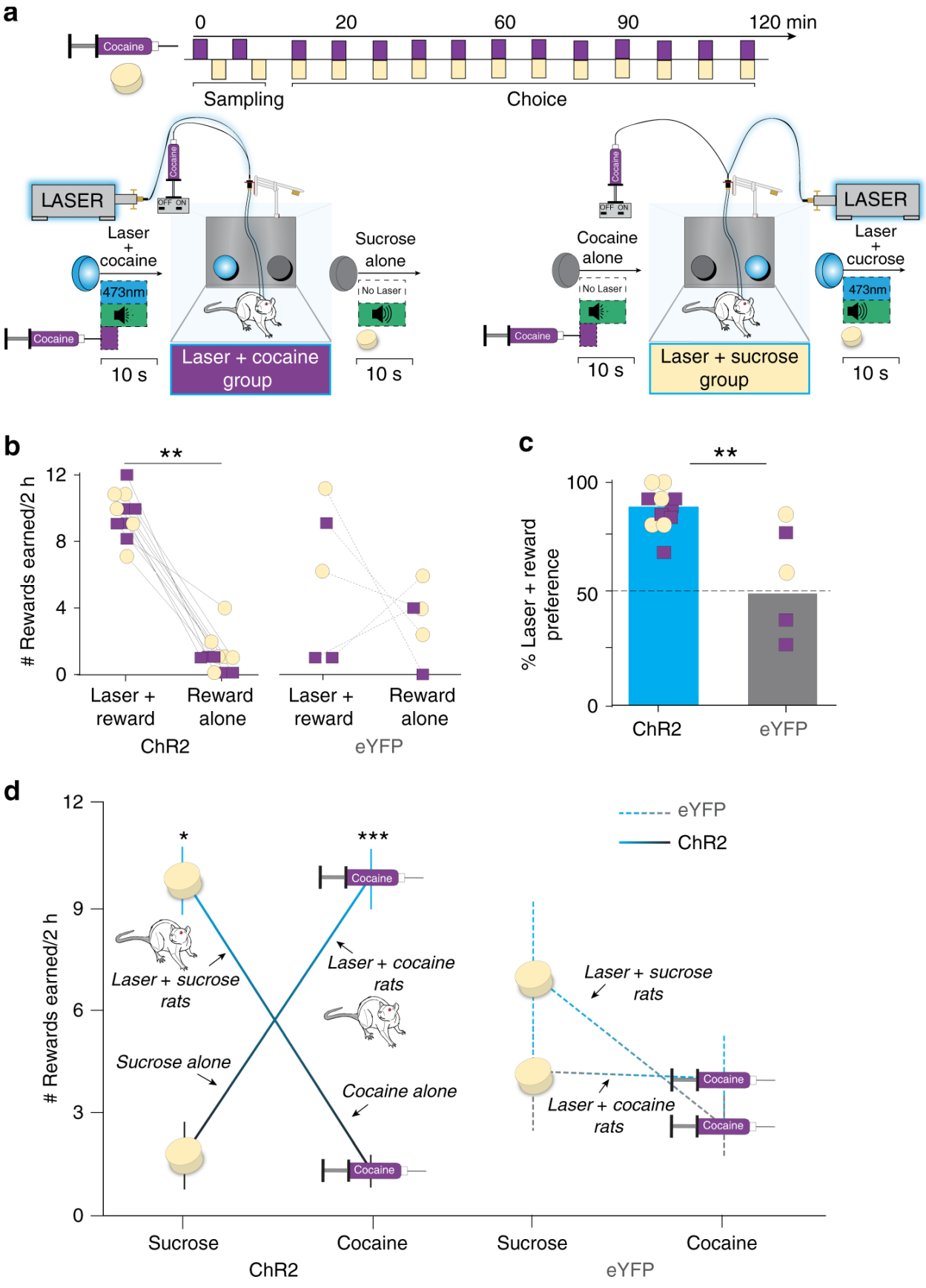


Figure 2.1 CeA ChR2 virus and Fos plumes.
a CeA photomicrograph (×10 magnification) shows green channelrhodopsin (ChR2) virus infection (AAV5-hSyn-ChR2-eYFP) and magenta Fos protein (N = 16 rats; ot: optic tract; scale bar: 0.5 mm). **b** Average Fos plume in CeA-mapped ChR2 rats after laser illumination (>200% above mean eYFP control baseline: light solid blue, >200% above normal unoperated tissue baseline: light blue dashed lines; >300% above eYFP: dark solid blue, >300% above baseline: dark blue dashed lines; D: dorsal, M: medial, L: lateral, V: ventral). See also Supplementary Fig. 2.1. **c** Mapped CeA sites of optic fiber implants for each ChR2 rat in cocaine/sucrose group and in shock-rod group (coronal view). Size of each site symbol reflects size of Fos plumes. Symbol colors in sucrose–cocaine group represents the percentage preference for individual's laser-paired sucrose/cocaine reward over alternative reward. For Shock-Rod Group, symbol color represents individual's number of shock-rod touches on day 3. Ic, internal capsule; GP, globus pallidus; CPu, caudate putamen; BLA, basolateral amygdala; IntC, intercalated amygdala; MeA, medial amygdala; BMA, basomedial amygdala.


a
0
20
60
90
120 min
Cocaine
Sampling
Choice
LASER
Laser + cocaine
473nm
Cocaine
10 s
Laser + cocaine group
Sucrose alone
No Laser
10 s
Cocaine alone
No Laser
Cocaine
10 s
Laser + sucrose group
LASER
Laser + cucrose
473nm
10 s
b
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Rewards earned/2 h
12
8
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100
50
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Cocaine
Laser + sucrose rats
Laser + cocaine rats
Sucrose alone
Cocaine alone
Cocaine
eYFP
ChR2
Laser + sucrose rats
Laser + cocaine rats
Cocaine
Cocaine
Sucrose
Cocaine
Sucrose
Cocaine
ChR2
eYFP

Figure 2.2 CeA ChR2 pairing controls pursuit of sucrose vs. cocaine.
a Sucrose–cocaine choice paradigm and timeline within an individual test session; nm, nanometers; s, seconds; min, minutes. **b** Earned rewards (via nosepokes on an FR1 schedule) during 2 h (h) sucrose vs. cocaine choice sessions. ChR2 rats, $N = 11$; eYFP rats, $N = 5$; two-way within and between subjects ANOVA, main effect of virus: $F_{1,10} = 4.6$, $p = 0.046$; ChR2 laser vs. nonlaser responses, two-sided paired *t*-test, $t_9 = 7.5$, $p = 0.000$, 95% CI: 4.8, 9.2, $d = 4.39$. Individuals with laser-paired sucrose in yellow circles and cocaine in purple squares. **c** Percent preference for laser-paired reward on day 4 (ChR2 rats: $N = 11$; eYFP rats: $N = 5$; two-sided unpaired *t*-test, $t_{10} = 3.4$, $p = 0.006$, 95% CI: 13, 60, $d = 1.81$). **d** Number of earned rewards for sucrose reward versus cocaine reward on last choice session, separated by reward type. CeA ChR2 laser + sucrose: $N = 5$ rats, sucrose vs. cocaine alone: two-sided paired *t*-test, $t_3 = 4.72$, $p = 0.04$, 95% CI: 1, 15, Cohen's $d = 5.11$; CeA ChR2 laser + cocaine: $N = 6$ rats, sucrose alone vs. cocaine: two-sided paired *t*-test, $t_5 = 7.7$, $p = 0.000$, 95% CI: 5.3, 9.2, $d = 4.6$). Control eYFP laser + sucrose: $N = 2$ rats, eYFP laser + cocaine: $N = 3$ rats; two-way repeated measures ANOVA, main effect of laser: $F_{1,3} = 1.35$, $p = 0.33$; h, hour. All data represent means and SEM. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

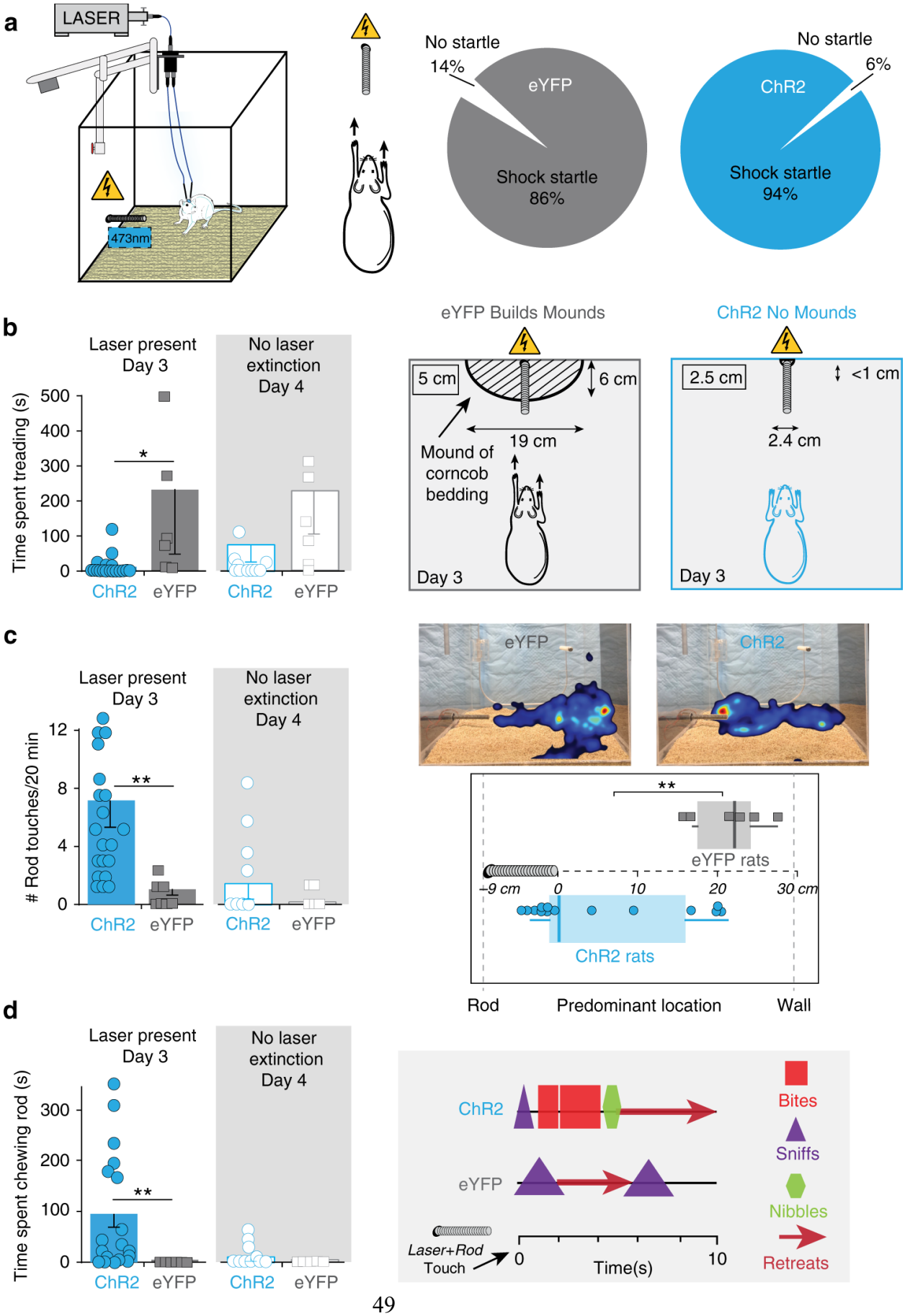
a
LASER
473nm
No startle
14%
eYFP
Shock startle
86%
No startle
6%
ChR2
Shock startle
94%
b
Laser present
Day 3
No laser
extinction
Day 4
Time spent treading (s)
500
400
300
200
100
0
*
ChR2
eYFP
ChR2
eYFP
eYFP Builds Mounds
5 cm
6 cm
19 cm
Mound of
corncob
bedding
Day 3
ChR2 No Mounds
2.5 cm
<1 cm
2.4 cm
Day 3
c
Laser present
Day 3
No laser
extinction
Day 4
Rod touches/20 min
12
8
4
0
**
ChR2
eYFP
ChR2
eYFP
eYFP
ChR2
**
eYFP rats
-9 cm
0
10
20
30 cm
ChR2 rats
Rod
Predominant location
Wall
d
Laser present
Day 3
No laser
extinction
Day 4
Time spent chewing rod (s)
300
200
100
0
**
ChR2
eYFP
ChR2
eYFP
ChR2
eYFP
Laser+Rod
Touch
0
Time(s)
10
Bites
Sniffs
Nibbles
Retreats

Figure 2.3 CeA paired stimulation creates attraction toward aversive shock rod.
a Apparatus: electrified shock rod protruded ~9 cm out from wall into plexiglas chamber. CeA laser illumination was paired with shock-rod encounters (when rat was within <2-cm proximity). Startle probability (i.e., paw withdrawal or jump) when touching the shock rod during the first encounter (ChR2, $N = 16$ vs. eYFP, $N = 6$ two-sided unpaired t-test: $t_{30} = 0.823$, $p = 0.42$). **b** Defensive treading on day 3 when laser was present (CeA ChR2 $N = 16$ vs. eYFP rats $N = 6$, two-sided unpaired t-test: $t_{21} = -2.4$, $p = 0.03$, 95% CI: 29, 410, $d = 0.72$) versus when laser was removed on day 4 ('No Laser Extinction', two-sided unpaired t-test: $t_{14} = -1.69$, $p = 0.16$). Drawing depicts defensive treading–burying behavior and consequent mounds of bedding. Placement and size of mounds shown by diameter of striped mound symbol (ChR2 vs. eYFP two-sided unpaired t-tests: height: $t_{26} = -4.3$, $p = 0.000$, 95% CI: −3.5, −1.2, $d = 1.73$; length: $t_{26} = -5.4$, $p = .000$, 95% CI: −6.9, −3.6, $d = 2.09$; width: $t_{26} = -6.5$, $p = 0.000$, 95% CI: −22.2, −11.6, $d = 2.34$). **c** Shock-rod touches when laser was paired with rod encounters (ChR2 rats, $N = 16$ vs. eYFP rats, $N = 6$, two-sided unpaired t-test: $t_{23} = 3.6$, $p = 0.002$, 95% CI: −11.8, −3.2, 11, $d = 1.07$), versus on no-laser day (two-sided unpaired t-test: $t_{11} = 1.18$, $p = 0.26$). Representative heatmaps (right) for individual rats shows location of representative rats during 20-min session (ChR2 or eYFP). Boxplots show predominant location: middle lines depict median (center), outer left lines extend to minimum value, outer right lines extend to maximum, and bounds of each box depict quartiles 2–3 (middle 50% of data; ChR2 rats, $N = 14$ vs. eYFP rats, $N = 6$, two-sided unpaired t-test: $t_{18} = -3.8$, $p = 0.001$, 95% CI: −25, −7.2, $d = 2.11$). **d** Time spent chewing shock rod with laser present (ChR2 rats, $N = 16$, eYFP rats, $N = 6$, two-sided unpaired t-test: $t_{18} = -3.3$, $p = 0.004$, 95% CI: −140, −30, $d = 1.06$) versus when laser was removed (two-sided unpaired t-test: $t_{11} = 0.9$, $p = 0.38$). Right panel shows microstructure choreograph of consummatory behaviors toward the shock rod from a representative ChR2 rat (top) and eYFP rat (bottom) during 10-s period following a shock rod touch. All data represent means and SEM. cm, centimeters. $*p < 0.05$, $**p < 0.01$.

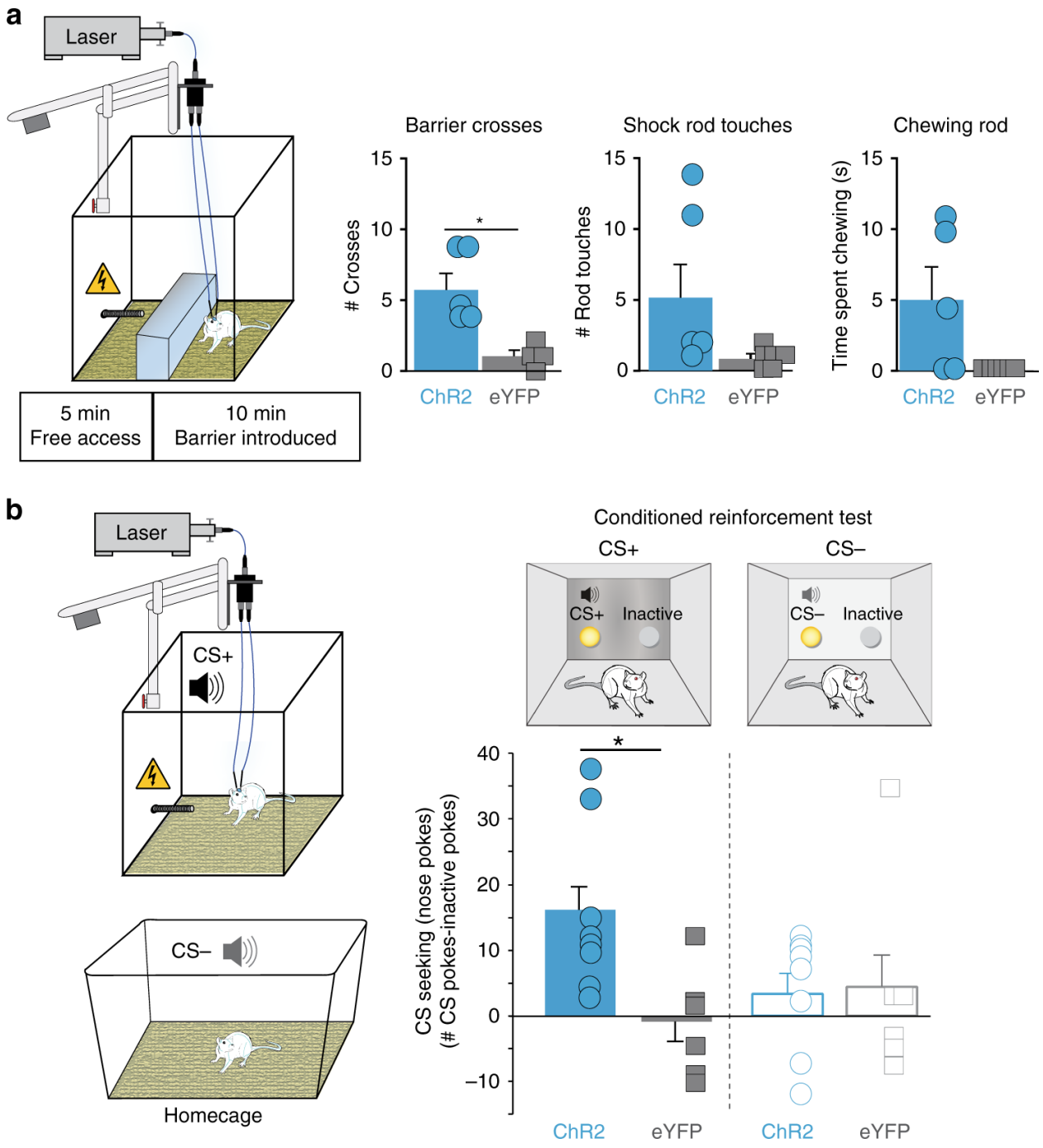

Figure 2.4 CeA ChR2 shock-rod attraction enhances shock-rod 'seeking' and cue incentive salience. **a** Apparatus shows novel opaque barrier interposed between rat and shock rod after 5 min into session. Number of barrier crosses to reach shock rod (ChR2 rats, $N = 5$ vs. eYFP rats, $N = 5$, two-sided unpaired t-test: $t_8 = 3.0$, $p = 0.02$, $d = 2.04$). Number of shocked touches on shock rod: two-sided unpaired t-test: $t_8 = 1.1$, $p = 0.08$. Time spent chewing on shock rod: two-sided unpaired t-test: $t_8 = 1.1$, $p = 0.08$. **b** Instrumental conditioned reinforcement test. Rats nosepoked to earn presentations of auditory conditioned stimulus (CS)+ previously paired with shock-rod encounters (top) or a different auditory CS− previously paired with homecage. Right graph depicts CS+ seeking as difference score between the number of nosepokes to earn CS+ sound (shock-rod-paired CS) over the number of nosepokes in inactive hole that

earned nothing. Left depicts CS− seeking: the number of nosepokes to earn CS− (homecage sound) sound over the number of nosepokes in inactive hole. CS+ vs. CS− were tested in separate sessions. Difference score for each day (CS nosepokes − inactive nosepokes) during CS+ and CS− sessions (ChR2 rats, $N = 8$, eYFP rats, $N = 6$; two-way repeated measures ANOVA, CS type × virus interaction: $F_{1,12} = 3.84$, $p = 0.04$; CS+: two-sided unpaired *t*-test: $p = 0.03$, 95% CI: 1.5, 29.6, $d = 1.35$; CS−: two-sided unpaired *t*-test: $p = 0.57$). Data represent mean and SEM. *$p < 0.05$.

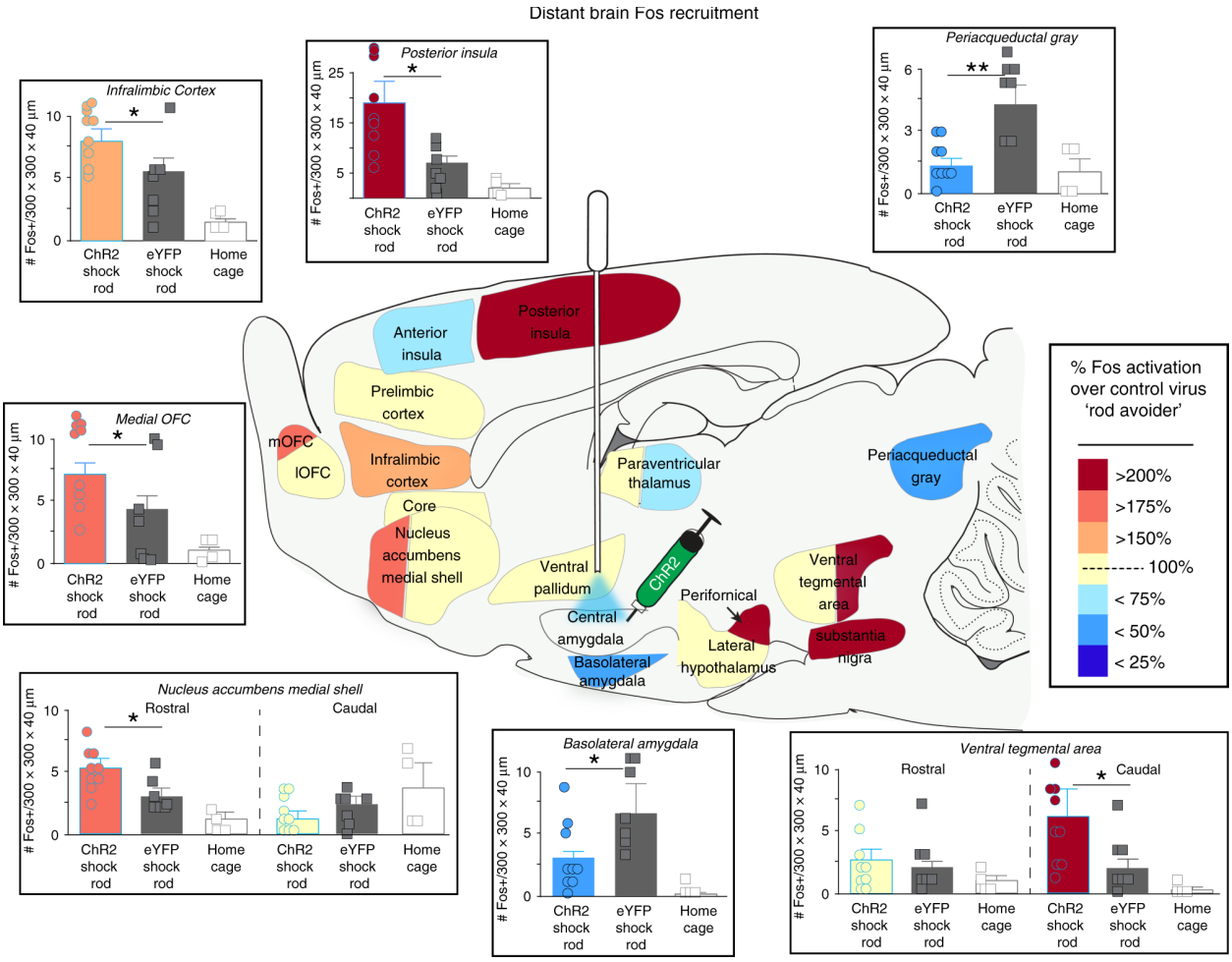

Figure 2.5 CeA ChR2 shock-rod attraction recruits mesocorticolimbic incentive circuitry. Brain map shows elevated Fos expression in recruited mesocorticolimbic structures in CeA ChR2 rats ($N = 9$, blue outline; colors denote % Fos ChR2 elevation immediately after a final exposure to shock rod compared to eYFP control rats ($N = 6$) and to homecage control baseline rats ($N = 4$). Cortical regions included medial orbitofrontal cortex (mOFC; one-way ANOVA between baseline homecage, eYFP, and ChR2 Fos: $F_{2,33} = 4.28$, $p = 0.02$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.025$), far-posterior insula (one-way ANOVA: $F_{2,33} = 4.28$, $p = 0.02$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.03$), and infralimbic cortex (one-way ANOVA: $F_{2,33} = 4.38$, $p = 0.02$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.012$). Subcortical structures included nucleus accumbens subregions: rostromedial quadrant of medial shell shown ($F_{2,33} = 4.96$, $p = 0.01$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.018$), caudal half of medial shell ($F_{2,33} = 0.58$, $p = 0.57$), core (NAc core (all subregions combined); $F_{2,33} = 0.2$, $p = 0.82$), ventral pallidum ($F_{2,33} = 0.57$, $p = 0.57$), perifornical area in lateral hypothalamus ($F_{2,33} = 3.13$, $p = 0.049$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.014$), basolateral amygdala ($F_{2,33} = 2.63$, $p = 0.04$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.012$), caudal ventral tegmental area ($F_{2,33} = 4.13$, $p = 0.02$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.012$), substantia nigra pars compacta ($F_{2,33} = 5.2$, $p = 0.002$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.02$), and periacqueductal gray ($F_{2,33} = 5.85$, $p = 0.007$, Bonferroni-corrected pairwise ChR2 vs. eYFP t-test: $p = 0.004$). Also see Supplementary Table 1. Bar graph data shown as mean number and SEM of Fos-expressing neurons in that structure per 300 × 300 × 40 micron (µm) sampling box. *$p < 0.05$, **$p < 0.01$.

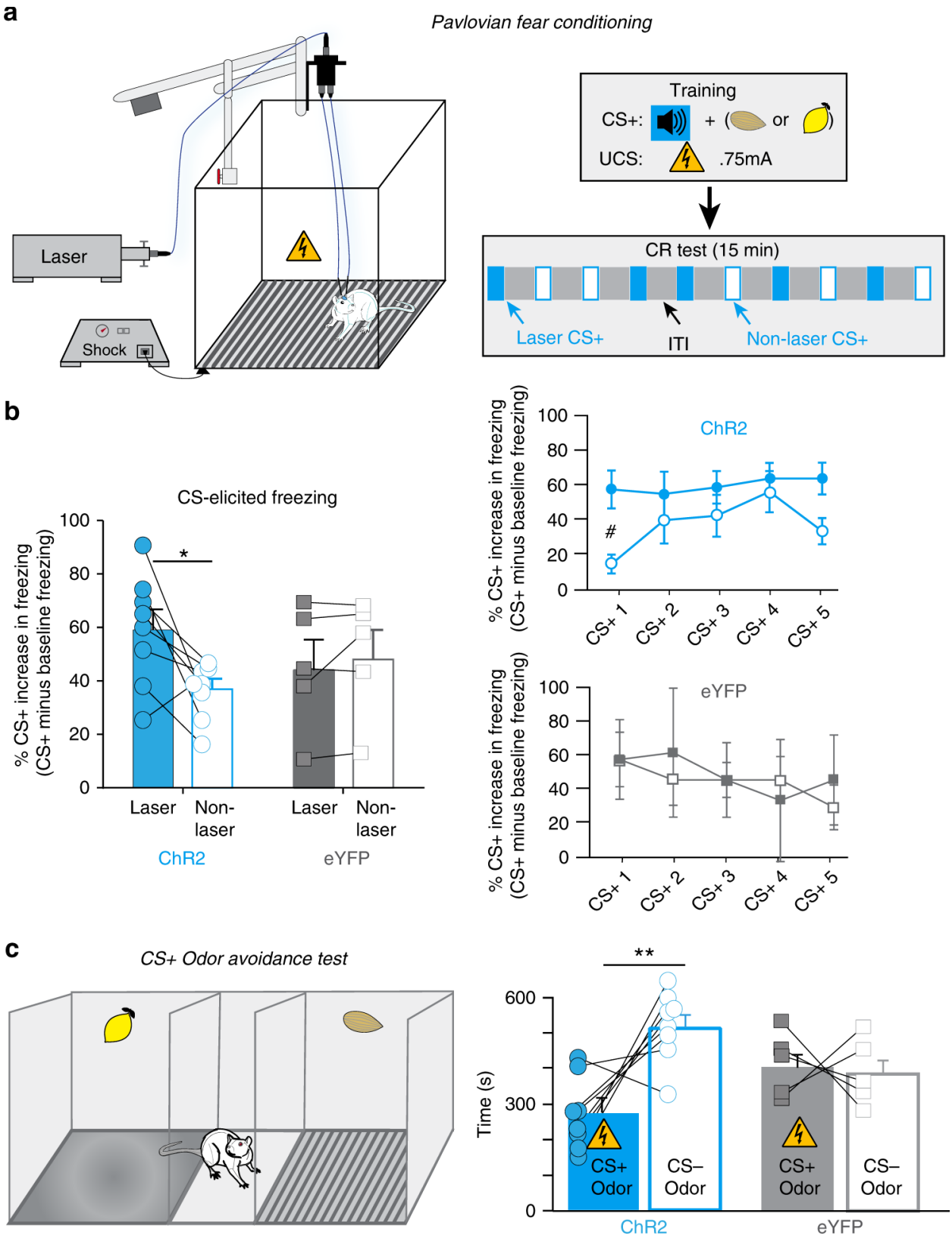

Figure 2.6 CeA stimulation during Pavlovian fear conditioning.

a Traditional Pavlovian fear conditioning paradigm: defensive freezing CR elicited by a CS+ tone that predicts unavoidable footshock UCS in Pavlovian chamber. Contextual odor CS+ (almond or lemon scent) was also paired with UCS shock chamber. Right shows training stimuli and session timeline. **b** Freezing conditioned response (CR) during subsequent test session without UCS (% CS-elicited freezing minus % baseline freezing; ChR2 rats, $N = 8$, eYFP rats, $N = 5$, two-way ANOVA, trial type × virus interaction: $F_{1,11} = 5.07$, $p = 0.036$; Bonferroni-corrected pairwise ChR2 laser vs. nonlaser trials *t*-test: $p = 0.035$). Right graphs show individual freezing CRs elicited by CS+ presentation over pre-CS+ baseline (ChR2: two-way repeated measures ANOVA, effect of laser: $F_{1,7} = 7.36$, $p = 0.03$, 1st trial laser vs. nonlaser freezing: Bonferroni-corrected pairwise *t*-test: $^{\#}p = 0.034$; eYFP: two-way repeated measures ANOVA, effect of laser: $F_{1,4} = 0.98$, $p = 0.38$). **c** Odor-place avoidance test, with separate compartments containing either CS+ odor (paired with footshock) or CS− odor (paired with homecage). Time spent in either CS+ or CS− side (CeA ChR2 rats, $N = 8$ vs. eYFP rats, $N = 5$, two-way ANOVA, virus × CS+ odor interaction: $F_{1,11} = 6.06$, $p = 0.03$, Bonferroni-corrected pairwise CS+ vs. CS− time, ChR2: $p = 0.04$, 95% CI: −393, −77, $d = -1.25$, eYFP: $p = 0.2$). Data represent mean and SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

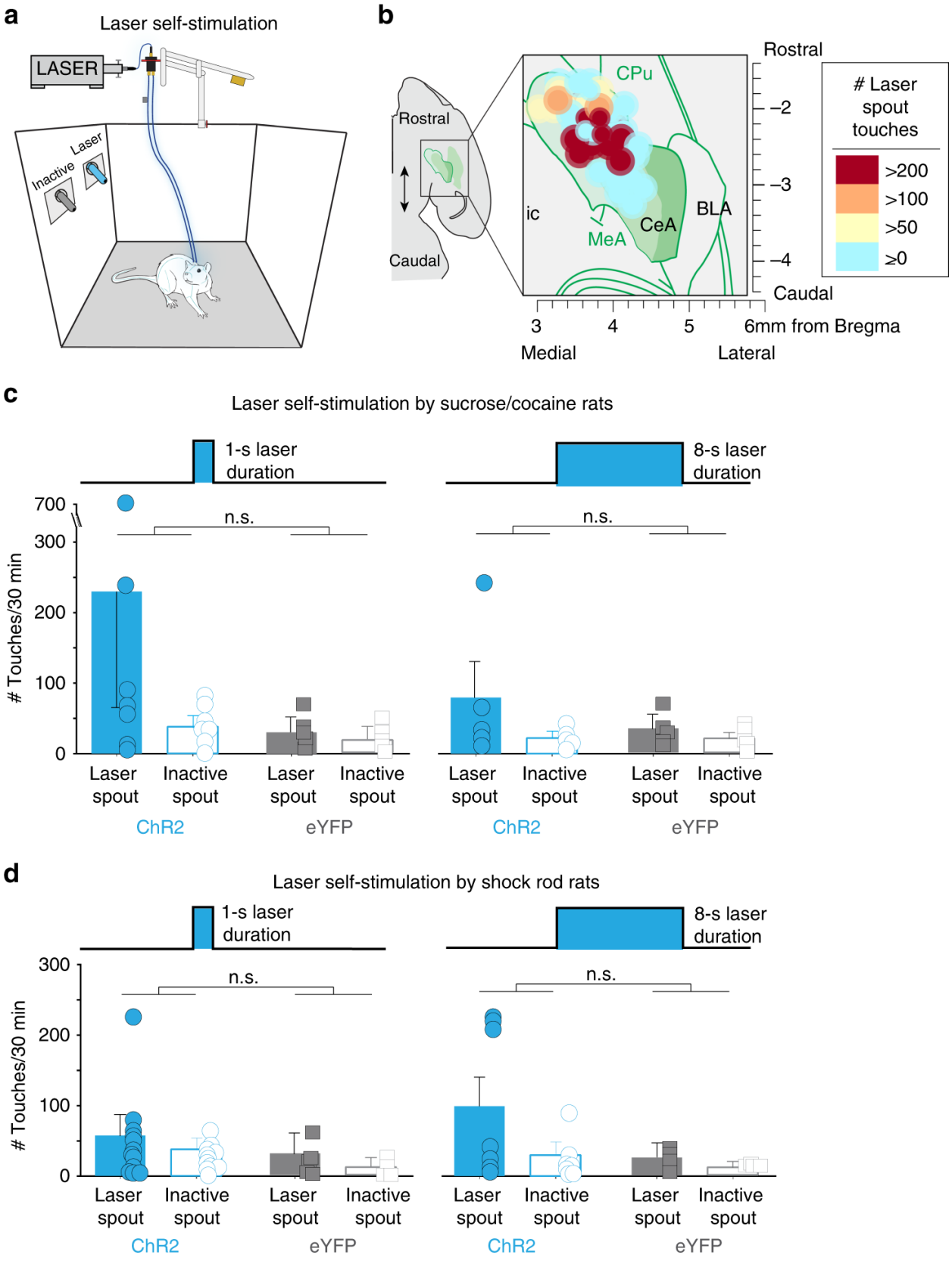

Figure 2.7 CeA ChR2 stimulation is unreliable as reinforcer alone.

A Laser self-administration task, in which touching a "laser spout" earned a laser stimulation (1-s or 8-s duration) and touching a separate "Inactive spout" earned nothing. **B** Placement map shows horizontal view of CeA optic fiber placements (size determined be average Fos plume from Fig. 2.1) with color of each placement indicating an individual rat's # of laser self-stimulations per 30-min test. **C** Total laser self-stimulations earned by rats from sucrose/cocaine experiments (ChR2 rats, $N = 10$ vs. eYFP rats, $N = 5$, 1 s laser duration, left: two-way ANOVA, virus × laser interaction: $F_{1, 15} = 1.71$, $p = 0.21$; 8 s laser duration, right: two-way ANOVA, virus × laser interaction: $F_{1, 15} = 2.06$, $p = 0.18$). **D** Total laser self-stimulations earned by rats from shock-rod experiments (ChR2 rats, $N = 18$, eYFP rats, $N = 6$, 1 s laser duration, left: two-way ANOVA, virus × laser interaction: $F_{1, 18} = 0.07$, $p = 0.8$; 8 s laser duration, right: two-way ANOVA, virus × laser interaction: $F_{1, 18} = 1.93$, $p = 0.18$). All data represent means and SEM; n.s., not significant.

CHAPTER III. Medial Amygdala Generates Incentive Motivation to Obtain Sucrose Reward and Attraction to Shock-Delivering Object in Some Rats.

Introduction

Medial amygdala (MeA) receives dense sensory projections from pheromone- and olfaction-processing regions in the brain and initially drove research into how MeA activity drives sociosexual behaviors (i.e., aggression, sex, parental care) (Bergan et al., 2014; Cooke & Woolley, 2005; Gomez & Newman, 1992; Kollack-Walker & Newman, 1997, n.d.; Newman, 1999). Continuations and extensions of these experiments went on to describe MeA receives projections regarding physiological states such as hunger. In one study it was found that hunger states in male mice alter agouti-related peptide (AgRP) incoming to MeA to act as a potential mechanism to flip between affective states. For example, after periods of starvation, incoming AgRP input can promote food-seeking, foraging behavior by reducing territorial aggression when starving and promoting territorial aggression when satiated (Padilla et al., 2016). In addition to physiological states, MeA neurons have been shown to undergo morphological changes to reflect past experiences such as previous sexual experience, aggression and parental experience (Chen et al., 2019; Y. Li et al., 2017; J. C. Nordman et al., 2020; Rasia-Filho, Fabian, Rigoti, & Achaval, 2004; Stolzenberg & Mayer, 2019; Zancan, da Cunha, Schroeder, Xavier, & Rasia-Filho, 2018). Within the extended amygdala, MeA is the medial counterpart to the CeA

and and has not yet been explored to be an effective generator of motivation as previously published in CeA. Importantly both MeA and CeA share a 'striatal-level' anatomical status when viewed through a macrosystem view of the forebrain. Our hypothesis is that MeA ChR2 will reveal a role of generating incentive motivation for rewards outside of a purely social context and be capable of narrowly generating motivation for a particular reward. These experiments will help place MeA on the map of incentive motivation and demonstrate a behavioral role for MeA as a striatal-like structure in the medial extended amygdala.

Materials and methods

Animals

Male and female Sprague Dawley rats (Females n=20, males n=12, at least 250g at the time of surgery; Charles River Laboratory) were group-housed in a 12h reverse light/dark cycle vivarium maintained at 21° degrees Celsius. Water and Purina chow pellets were always available ad libitum. All experimental methods and procedures were approved by the Institutional Animal Care and Use Committee at the University of Michigan.

Surgery

Prior to surgery, all rats (n=32) were handled by experimenters for approximately 30 minutes each day for several days to become accustomed to human handling. Prior to surgery, anesthesia was induced in rats with 5% isoflurane gas (Henry Schein, Wixom, MI) and maintained with 1-2% isoflurane throughout the duration of the surgery. Rats also received an intraperitoneal injection of atropine prior to surgery (Sparhawk Laboratories, Shawnee Mission, KS; 0.04mg/kg)

to reduce accumulation of fluid in the lungs, as well as injections of isotonic saline (3mL) to prevent dehydration, cefazolin (Henry Schein, Wixom, MI; 100mg/kg) to prevent infection, and carprofen (Henry Schein; 5mg/kg) as postsurgical analgesic. Rats were placed in a stereotaxic device (David Kopf Instruments) to receive bilateral infusions of virus targeted at the medial nucleus of amygdala (MeA; see Fig. 3.1, 3.2A). Virus was either the excitatory optogenetic virus (ChR2), AAV5-hSyn-ChR2-eYFP (n=15), or an optically inactive control virus (eYFP), AAV5-hSyn-eYFP (n=6). A volume of 0.75µLs of virus was microinjected bilaterally into each medial amygdala, at a rate of 0.1µL per minute. After the microinfusions, the microinjector was left in place for an additional 10 minutes to allow for virus diffusion from the injection site. As a separate anatomical control group, other rats (female n=5, male n=1) received ChR2 virus bilaterally into the basomedial nucleus of the amygdala, a 'cortical-like' structure in macrosystem frameworks (LW Swanson, 2005; LW Swanson, 2000; LW Swanson and Petrovich, 1998) that provides inputs to the medial amygdala. This basomedial nucleus was chosen as anatomical control site because previous studies of incentive motivation induced by optogenetic stimulation of the central nucleus of the amygdala (CeA) found that stimulation of CeA's own cortical-type input nucleus, the basolateral nucleus (BLA), failed to induce incentive motivation and so served as a within-amygdala anatomical control site. Bilateral optic fibers (200µm) were implanted 0.3mm dorsal to virus injection site in MeA on both sides of the brain in the same surgery, or above the basomedial nucleus control site. Rats received two additional injections of carprofen at 24hr and at 48hr after surgery for pain management. Rats were allowed to recover for 3 weeks, to allow time for virus expression, before behavioral training and testing began. A total of five rats were excluded from analyses due to loss of headcap (n=3) and intense seizures induced by MeA ChR2 (n=2).

Instrumental two-choice sucrose task

To assess whether optogenetic stimulation of medial amygdala could control motivation for a paired sugar reward, rats underwent instrumental training in a two-choice sucrose task, identical to that used in a previous optogenetic study of motivation induced by CeA stimulations (M. J. F. Robinson et al., 2014). First, rats were habituated to the Med Associates chamber (30.5 x 24.1 x 21.0 cm) and underwent one day of magazine training. Next, in 9 instrumental daily training sessions (30 min each) rats had access to two retractable levers, each of which could be inserted into the chamber or withdrawn during the session. One lever was designated '*Sucrose+Laser',* and presses on it earned delivery of sucrose pellets (45mg; Test Diet) plus bilateral MeA laser illumination (8 sec duration, onset at final lever press that earned a sucrose pellet; 473nm; 1-3mW; 25Hz [15ms on, 25ms off]) and simultaneously a distinctive auditory cue (8 sec of either tone or white noise; randomly assigned across rats to levers; but always consistent for the same lever for a given rat). The other lever was designated '*Sucrose Alone',* and presses on it earned an identical sucrose pellet alone, without any laser, plus its own distinctive 8-sec auditory cure (white noise or tone). Lever assignments always remained the same for a given rat, but were counter-balanced across rats. A third lever was always present on the opposite wall of the chamber, but never earned any outcome, and simply served as a control measure to detect non-instrumental presses due to general activity.

All instrumental training and test sessions began with presentation of a single lever, either *Sucrose+Laser* or *Sucrose Alone* (counter-balanced across trials). When the rat successfully earned that lever's outcome (e.g., *Sucrose+Laser*), the first lever was withdrawn and the alternative lever was presented until the other outcome was earned (e.g., *Sucrose Alone*). The

single-lever-at-a-time cycle was repeated once more, so the rat earned a second outcome from each lever. These single-lever presentations were done to ensure the rat sampled from both levers, to remind it of the different outcomes, prior to being allowed to choose between both levers simultaneously present for the rest of the session. An 8 second timeout period followed each sucrose pellet earned by a lever during 2-choice trials, during which further presses on it earned nothing, but rats could still earn the alternative reward outcome by switching to the other lever during that 8 second timeout.

Rewards were earned on a fixed ratio 1 press (FR1) schedule for four days, then on a fixed ratio 4 schedule (FR4) for one day, a random ratio 4 (RR4) for one day, and random ratio 6 schedule (RR6) for the remaining three days. Additionally rats needed to successfully earn at least 25 rewards per day at each schedule before advancing to the next schedule; any that failed to receive at least 25 rewards repeated the current schedule the next day until they met criterion for advancing.

Laser-alone reinforcement during sucrose extinction?

Subsequently, a subgroup of the rats described above (n=3) underwent four daily 30-min sucrose-extinction sessions, in which lever presses on the *Sucrose+Laser* lever earned the 8-sec presentation of MeA laser (473nm; 25Hz; 1-3mW) plus its customary auditory cue but no longer resulted in the delivery of a sucrose pellet. Similarly, presses on the *Sucrose Alone* lever earned nothing except for the customary auditory cue. This was done to assess reinforcement efficacy of MeA laser and conditioned reinforcers, and specifically to ask if MeA laser by itself was sufficient to maintain instrumental responding, once established by laser pairing with sucrose.

Progressive Ratio

After the two-choice sucrose, MeA ChR2 rats (n=10; MeA eYFP control n=7) that did not undergo sucrose extinction underwent two days of progressive ratio 'breakpoint' tests of instrumental effort, in order to separately assess if MeA laser intensifies incentive motivation to obtain its paired reward. On each day, rats had access to only one of the two levers for the entire 30 minute test. On one day, only the *Sucrose+Laser* lever was available, and earned its usual sucrose paired with8-sec MeA laser and distinctive auditory cue. On the other day, only the *Sucrose-Alone* lever was available, and earned its usual sucrose and auditory cue without laser (order of *Sucrose-Alone* vs *Sucrose+Laser* days counterbalanced across rats). In both sessions the number of lever presses necessary to earn the next reward increased in an exponential, progressive ratio schedule after each sucrose pellet = 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77… 178, 219, 268… , based on a progressive ratio formula of PR= [5e=^reward number*0.2]-5 (Richardson and Roberts, 1996; Saunders and Robinson 2011; Robinson et al. 2014; Warlow et al., 2017). This design was selected to measure effort breakpoint or the maximum price the rat is willing to pay as cost rises.

Laser Self-Stimulation

To more directly probe potential reinforcing effects of MeA ChR2 excitation, laser self-stimulation (without sucrose) was assessed in the same rats from above using an active spout-touch self-stimulation task. MeA ChR2 rats (females n=6, male n=1) were tested for laser self-stimulation after the sucrose choice and breakpoint tests described above, and a subset of these rats was also tested for laser self-stimulation prior to any sucrose training (n=5 females; in order

to assess whether laser self-stimulation propensity was altered by having laser associatively paired with sucrose in the interim).

In the spout-touch self-stimulation task, rats were placed into Med Associates operant chambers equipped with two novel and empty sipper spouts on the back wall of the chamber, positioned ~12 cm apart. A metal grid floor was wired to close a circuit to detect contacts at each spout. Each touch on one spout (designated as 'active spout'; spout assignment counterbalanced between rats) delivered MeA laser stimulation [(473nm; 25Hz; 1-3mW; bin duration 1 or 8 sec); FR1 schedule]. Some rats always earned a 1 s pulse (n=7; females n=6 and male n=1) and others always earned an 8 s pulse (n=5; females n=3 and males n=2); no auditory cue]. The 1 s pulse was used because it has supported optogenetic self-stimulation in previous studies (Kravitz et al., 2012; Witten et al., 2011). The 8 s pulse was used because it was identical to that used in the instrumental 2-choice sucrose task above. Touching the other available spout produced no consequence, and simply served as a control measure for touches due to exploration and general motor activity. Each test session lasted 30 minutes and was repeated on three separate days.

Shock rod test sessions

MeA ChR2 rats (male n=7; female n=3) and eYFP controls (male n=1; female n=1) underwent three 20 minute test sessions in which they received laser excitation (15 ms on, 25 ms off (25Hz); 2-3mW) each time they came within 2cm of the shock rod. The intensity of the shock received ranged from 0.1 to 0.55mA as measured by an ammeter recording 17 total contacts from 7 different rats. The fourth day was a laser extinction session in which close proximity to the rod no longer triggered MeA ChR2. For longer description of chamber and methodology see pg 18 of Chapter II shock rod methods (Warlow, Naffziger, & Berridge, 2020).

Pavlovian fear conditioning

Following completion of the four shock rod sessions, rats (MeA ChR2 n=10, eYFP n=2) underwent Pavlovian fear training across three days. Test sessions were run identical to those described in the previous chapter on pg 20 (Warlow, Naffziger, et al., 2020).

Brain Histology

After the completion of experiments, rats were deeply anesthetized with an overdose of sodium pentobarbital (150-200 mg/kg) and transcardially perfused. Brains were extracted and post-fixed in 4% paraformaldehyde for 48 hours before being transferred into a 25% sucrose solution and stored at 4°C. Brains were then sectioned into 40 μm coronal sections and cryoprotected until ready to be mounted, cover slipped with ProLong Gold anti-fade reagent (Invitrogen). Images taken with a Leica microscope (Leica Microsystems, Inc., Buffalo Grove, IL) at 10x and 40x magnification were marked in Adobe Illustrator (RRID: SCR:014198) on a rat brain atlas (Paxinos & Watson, 2007). For each MeA site, nine images (3 X 3; 10X magnification) were compiled using Oasis Surveyor software (Objective Imaging, Inc., Kansasville, WI; RRID: SCR: 014433) into one single image centered on the fiber tip.

Statistical Analysis

Data was analyzed utilizing the software SPSS to run repeated-measure ANOVAs and t-tests with Bonferroni corrections. For non-normally distributed data including all progressive ratio tests, Friedman's two-way ANOVAs were used as nonparametric within-subject tests and Kruskal-Wallis one-way ANOVAs for between subject tests, followed by Wilcoxon sign-ranked/

Mann-Whitney tests for individual comparisons. Effect sizes for parametric tests were calculated using Cohen's d and for nonparametric tests using $r = \frac{Z}{\sqrt{N_1+N_2}}$. For all analyses, the significance level was set at $p < 0.05$, two-tailed.

Results

MeA ChR2 induces preference for laser-paired sucrose in 2-choice test

MeA ChR2 rats nearly exclusively pursued their *Sucrose+Laser* option, paired with 8-s laser stimulations that began with onset of the final lever press that earned a sucrose pellet, by a 6:1 ratio over the *Sucrose Alone* option (for *Sucrose+Laser*; $F_{(1,15)}$=6.29, p<.000; Fig. 3.3). The MeA ChR2 *Sucrose+Laser* preference emerged by Day 2day at 3:1 ratio, rose to 4:1 ratio on Day 3, and reached roughly 6:1 ratio for the final days 7-9 (Day 1 $t_{(8)}$=2.1, p=.072; Day 2 $t_{(8)}$=2.3, p=.049; Day 3 $t_{(8)}$=2.5, p=.034). For example, MeA ChR2 rats made ~371 ± 75 presses on average for the *Sucrose+Laser* lever during days 7-9 compared to only ~56 ± 33 responses per day on the *Sucrose Alone* lever (Day 7 $t_{(8)}$=4.76, p=.001; Day 8 $t_{(8)}$=3.8, p=.005; Day 9 $t_{(8)}$=3.72, p=.006). Since laser assignment to the two lever locations in the chamber was balanced across rats, physical lever location was not a significant factor in driving MeA ChR2 preference for -*Sucrose+Laser* option ($F_{(1,14)}$=1.007, p=0.333).

By contrast, eYFP control rats with optically-inactive virus initially chose equally between the two options, and eventually developed avoidance of the *Sucrose+Laser* option, and instead a 2:1 preference for the *Sucrose Alone* option by days 7-9 ($F_{(1,6)}$=5.12, p=0.64); Day 7 $t_{(6)}$=-1.31, p=.237; Day 8 $t_{(6)}$=-2.1, p=.08; Day 9 $t_{(6)}$=-3.0, p=.024), presumably due to potentially aversive effects of light and heat in the medial amygdala ($F_{(1,14)}$=20.76, p<.000). A separate anatomical control group of rats had ChR2 virus and optic fiber sites in the basal medial amygdala (BMA ChR2 rats), which is slightly ventral and lateral to MeA. These BMA ChR2 control rats, chose equally between *Sucrose+Laser* and *Sucrose Alone* options throughout all 9 days ($F_{(1,8)}$=.36, p=.94). Thus MeA preference/avoidance effects here appeared to be relatively specific to the

medial nucleus of amygdala itself and did not spread to a more ventrolateral amygdala subregion.

Both MeA ChR2 rats and eYFP rats increased instrumental working for sucrose across the 9 days ($F_{(8,7)}$=9.19, p=.004), and control eYFP rats showed a trend towards the greatest increases ($F_{(8,7)}$=3.37,p=0.064), reaching the highest level of >600 total presses on both levers combined during the last two days of the two-choice sucrose task [(# Cumulative Lever Presses day 8 ChR2 405.4 ± 58.7 vs eYFP 643 ± 88.2, $t_{(15)}$=-2.34, p=.034), (# Cumulative Lever Presses day 9 ChR2 392 ± 76.2 vs eYFP 692 ± 94, $t_{(15)}$=-2.5, p=.025)]. Consequently, control eYFP rats earned about 50% more sucrose pellets in total (eYFP day 8 85.8 ± 10.7 pellets; day 9 81.7 ± 9.8) than MeA ChR2 rats (ChR2 day 8 52.8 ± 6.5 pellets; day 9 47.1 ± 7.8; day 8 $t_{(13)}$=-2.81, p=.015; day 9 $t_{(13)}$=-2.8, p=.017).

Is MeA ChR2 laser sufficient to maintain pursuit in absence of sucrose?

Does MeA ChR2 preference for *Sucrose+Laser* reflect the value of laser per se adding together with sucrose value, or instead an interactive MeA ChR2 magnification of the value of sucrose? To answer that, after self-stimulation tests, MeA ChR2 rats were retrained for several days retraining on the 2-choice sucrose task to re-establish previous levels of responding. Then a subsequent 'sucrose-extinction' condition was imposed in which pressing the designated lever continued to earn laser illuminations but sucrose pellets were no longer earned by either lever. In this condition, the former *Sucrose+Laser* lever now earned Laser alone, and the former Sucrose Alone lever now earned nothing. This assessed if MeA laser by itself could at least *maintain* levels of preferential instrumental responding that were already established by the combination of *Sucrose+Laser.* Results showed that, in the absence of sucrose rewards, MeA

ChR2 rats no longer demonstrated a detectable preference for their *Sucrose+Laser* lever ($F_{(1,4)}$=16.23, p=.016; Fig. 3.5).

Beginning on the first day sucrose was discontinued, MeA ChR2 rats reduced their responding on the former *Sucrose+Laser* lever by over 85%, dropping from ~573 (±179.3) to ~80 (±12.5) presses on the lever that now earned Laser alone ($t_{(2)}$=2.59, p=.122). The rats no longer displayed a significant preference for the laser-delivering lever over the alternative lever that now delivered nothing ($t_{(2)}$ =1.82; p=.21). By the 3rd day of sucrose-extinction MeA ChR2 rats pressed the *Laser alone* lever (former *Sucrose+Laser* lever) only ~8.3 (±7.8) times, earning only 1.7 (±1.2) laser activations of MeA, and pressed the *Nothing lever* (former *Sucrose Alone* lever) ~7 (±4.2) times. This indicates that MeA illuminations by themselves were able to maintain the strong preference or elevated levels of instrumental responding that had previously been achieved by the *Sucrose+Laser* combination. That failure suggests that MeA ChR2 stimulation primarily amplified the incentive value of its paired sucrose option to control sucrose pursuit in the 2-choice task, rather than adding a separate stable incentive value that is powerfully sought on its own.

Incentive motivation: breakpoint elevation

In a progressive ratio task intended to assess MeA ChR2 effects on the intensity of incentive motivation to obtain sucrose reward, MeA ChR2 stimulation appeared to increase incentive motivation breakpoint for sucrose. MeA ChR2 rats pressed ~180% more on the single available lever that earned sucrose rewards in their *Sucrose+Laser* session than in the *Sucrose Alone* session than they worked for sucrose on another day when sucrose was earned alone without laser (*Sucrose+Laser* day lever presses ~370 ± 32.6 vs *Sucrose Alone* ~204 ± 18; $t_{(9)}$=7.7,

p<.000; see Fig. 3.4), and effort breakpoint rose from 56 ± 13 in the *Sucrose Alone* session to 94 ± 23 in the *Sucrose+Laser* session; ($t_{(9)}$=3.62, p=.006). On the *Sucrose+Laser* day, MeA ChR2 also pressed more than control MeA eYFP rats did ($F_{(1,16)}$ =6.63, p=.021), although the corresponding difference in breakpoints did not reach significance between ChR2 and eYFP groups (MeA ChR2 94 ± 23 vs eYFP 45.6 ± 10.8; $F_{(1, 16)}$ =2.8, p=.12). On the *Sucrose Alone* day without laser, MeA ChR2 and eYFP groups both pressed a similar number of times (MeA ChR2 lever presses 204 ± 18 vs eYFP 317 ± 87; $F_{(1,16)}$=2.26, p=.15). By comparison, control MeA eYFP rats with inactive virus did not differ in their total number of presses or breakpoints on *Sucrose+Laser* day vs *Sucrose Alone* day (*Sucrose+Laser* lever presses 212 ± 57 vs *Sucrose-Alone* 317 ± 87 (t_6=-1.94, p=.101); *Sucrose+Laser* breakpoint reached 45.6±10.8 vs *Sucrose-Alone* 63.6 ± 10 (t_6=-1.68, p=0.14)).

MeA inhibition by iC++ induces avoidance of Sucrose+Laser option

A separate group of rats received MeA viral microinjections of the inhibitory Cl- ion channel ChR2 variant iC++ (MeA iC++ group). In the two-choice sucrose task, MeA iC++ rats developed a late-emerging avoidance of their *Sucrose+Laser* option that became significant on days 8-9, and instead preferred *Sucrose Alone* over *Sucrose+Laser* by a 4:1 ratio (*Sucrose Alone* 271 ± 50 lever presses; *Sucrose+Laser* ~60 ± 37 vs $t_{(2)}$=-15.67, p=.041; see Fig. 3.6). However, MeA iC++ rats still earned roughly the same number of sucrose rewards in total during days 8-9 as MeA ChR2 rats, suggesting that MeA iC++ inhibition did not detectably suppress overall sucrose seeking (last day of testing MeA iC++ earned ~41 ± 11 sucrose pellets vs. MeA ChR2 ~47 ± 7 pellets, $F_{(1,10)}$=.150, p=.708).

1-sec laser bins (but not 8-sec) support spout-touch self-stimulation

In a test of whether MeA ChR2 laser excitation was an incentive stimulus by itself, MeA ChR2 rats were tested for potential laser self-stimulation in an instrumental spout-touch task (MeA ChR2 n=11, eYFP n=9). Rats could earn brief bins of laser illumination (either 1 sec or 8 sec bins; each rat tested on both durations in counter-balanced order) by touching one of two available empty metal spouts (designated as *active spout*). The other spout delivered nothing and served as a control measure for general exploratory touches All MeA ChR2 rats were tested for laser self-stimulation after the 2-choice sucrose testing, and a subset of MeA ChR2 rats (n=4) had additionally been pre-screened for laser self-stimulation prior to sucrose training in case the experience of having sucrose associatively paired with MeA laser stimulation alters the reinforcing qualities of MeA ChR2 excitation by itself.

1-sec MeA ChR2 supports self-stimulation

Initial laser self-stimulation: Prior to being trained in the 2-choice sucrose task, a subgroup of MeA ChR2 rats (n=4) were tested for initial 1-sec laser self-stimulation on the spout-touch self-stimulation task (473nm; 15ms on, 25 ms off; 1-3mW). For these rats, MeA ChR2 laser supported robust initial self-stimulation ($F_{(1, 6)} = 11.943$, p=.014; see Fig. 3.7).

Subsequent laser self-stimulation: A group of 7 MeA ChR2 rats (including the 4 initially pre-screened) were tested more thoroughly for laser self-stimulation after 2-choice sucrose task ended. For 1-sec laser bins, 5 of 7 MeA ChR2 rats met criteria for robust laser self-stimulation (including the male MeA ChR2 rat), defined as >50 laser self-stimulations per 30 minute session, and twice as many contacts to active spout as on inactive spout on test day 1, ranging from >50 to >800 touches on the laser-delivering spout, and more than eYFP control rats (total 3-days $F_{(1,}$

$_{13)}$ =5.514, p=.035). On the first day, MeA ChR2 rats earned 139 ± 63 self-stimulations compared to only 44 ± 12 touches on the inactive spout (Wilcoxon Signed Ranks Z=-1.57, p=.116). On the second day, the entire group of MeA ChR2 earned 168 ± 111 self-stimulations vs 27 ± 10 touches on inactive spout (U=7, p=.025; Wilcoxon Signed Ranks Z=-1.992, p=.046). On day 3 MeA ChR2 rats earned 280 ± 120 laser self-stimulations compared to only 30 ± 167 touches on the inactive spout (U=4, p=.009; Wilcoxon Signed Ranks Z=-1.992, p=0.046). The 5 of 7 MeA ChR2 rats meeting robust >50 criteria for self-stimulation for 1-sec laser showed even higher rates, starting on day 1 with 214 ± 51 active touches compared to 41 ± 12 inactive contacts, 220 ± 132 active contacts and 36 ± 12 inactive touches on day 2, to 302 ± 139 active touches and 37 ± 18 inactive touches on day 3. Laser-spout preference was not simply due to location in the chamber, which was counterbalanced in laser assignment across rats, and physical spout position was not a significant factor ($F_{(2, 9)}$ =1.558, p=0.262). The 4 MeA ChR2 rats that were initially pre-screened remained robust self-stimulators, earning about 70 laser self-stimulations per session both before and after 2-choice sucrose training (before= ~73 ± 5; after = ~71 ± 6%, $F_{(1,6)}$=0.125, p=0.74).

Consequently, as an entire group, MeA ChR2 rats self-stimulated for 1-sec illuminations far more than eYFP control rats ($F_{(1,13)}$=5.51; p=.035). Control eYFP rats on day 1 earned 35 ± 21 self-stimulations and similarly made 36 ± 36 inactive spout contacts ($t_{(8)}$ =-0.337, p=.745). On day 2 eYFP earned 20 ± 24 active illuminations on day 2 vs 35 ± 36 contacts on inactive spout ($t_{(8)}$ =-1.92, p=.092), and on day 3 made 33 ± 9 active contacts and 43 ± 14 inactive contacts (Wilcoxon Signed Ranks Z=-1.304, p=.192). MeA ChR2 rats showed a growing preference for the active spout across the three test days (~61 ± 11% on day 1, ~70 ± 9% on day 2 and ~80 ±

7% by day 3), whereas eYFP rats made roughly equal contacts on both spouts across all days (~41 ± 9% on day 1, ~41 ± 7% on day 2, and 50 ± 7% on day 3).

The two remaining MeA ChR2 rats both failed to meet either the robust criterion or weaker criterion for self-stimulation on day 1 (>10 contacts and twice as many contacts to the laser-delivering spout as inactive spout), made only 8 ± 6 self-stimulations on Day 1, 9.5 ± 1.5 on day 2, and 3 ± 1 on day 3. Thus these 2 of 7 MeA ChR2 rats failed to show laser self-stimulation despite having anatomical sites and virus infection similar to weak and robust self-stimulators. However, these two MeA ChR2 rats that failed to self-stimulate laser by itself had shown *Sucrose+Laser* preferences in the two-choice task that were comparably strong (approximately 6:1 preference for *Sucrose+Laser*) to those of the 5 MeA ChR2 rats that did robustly self-stimulate (approximately 5:1 for *Sucrose+Laser*). While there was a correlation with preference for *Sucrose+Laser* during operant behavior with propensity to self-stimulate at 1 second ($r=0.79$, $p=0.036$), MeA ChR2 self-stimulators are likely not entirely responsible for driving the sucrose preference observed in the two-choice sucrose task above as MeA ChR2 rats that failed to self-stimulate showed identical *Sucrose+Laser* preference across sucrose training to those that did self-stimulate ($F_{(1, 8)} =0.553$, $p=0.48$). Similarly, across MeA ChR2 self-stimulators and nonself-stimulators there was no overall difference in responding for *Sucrose+Laser* on the last day of sucrose training (Self-Stimmers made 435 ± 139 to *Sucrose+Laser* lever and Nonself-Stimmers made 303 ± 26; $F_{(1, 9)} =0.581$, $p=0.145$) and no difference in breakpoint reached when working for *Sucrose+Laser* reward (breakpoint completed by self-stimulators was ~81 ± 6 compared to those not meeting self-stimulation criteria 56 ± 6). Upon closer inspection, the rat that demonstrates the highest number of laser-delivering spout contacts during self-stimulation also lever pressed more than any other MeA ChR2 rat.

8-second MeA ChR2 laser fails to support self-stimulation in most rats

Only 1 of 5 MeA ChR2 rats met robust >50 criteria for self-stimulation, beginning on day 2 (170 self-stimulations vs 42 inactive spout touches), and persisting on day 3 (158 self-stimulations vs 8 inactive spout touches). By contrast to their self-stimulation at 1-sec duration, 4 of the same 5 MeA ChR2 rats failed to self-stimulate for longer 8-sec bins of illumination, although 8-sec had been the duration that controlled their sucrose pursuit in the 2-choice task and breakpoint task ($F_{(1,4)}$=3.92, p=.12; see Fig. 3.7).

Overall, MeA ChR2 rats as an entire group failed to show significant laser self-stimulation for 8-sec bins than the eYFP control group ($F_{(1,6)}$=1.579, p=.256; ~23 ± 14 self-stimulations vs ~28 ± 16 inactive spout contacts on day 1 ($t_{(4)}$=-.382, p=0.72), 45 ± 30 self-stimulations vs. 11 ± 8 inactive contacts on day 2 ($t_{(4)}$=1.49, p=.21), and 51 ± 28 self-stimulations vs.16 ± 13 inactive spout contacts on day 3 ($t_{(4)}$=1.21, p=.29)). Control eYFP rats similarly failed to self-stimulate at 8-sec laser durations (43 ± 13 self-stimulations vs 48 ± 19 contacts on inactive spout on day 1 ($t_{(5)}$ =-0.26, p=0.81); 38 ± 18 self-stimulations vs ~33 ± 11 inactive contacts on day 2 ($t_{(5)}$ =0.25, p=0.82); 30±10 self-stimulations vs ~33 ± 9 inactive contacts on day 3 ($t_{(4)}$ =0.32, p=0.77).

Comparing the one MeA ChR2 rat that did self-stimulate at 8-sec on the 2-choice sucrose task duration to the MeA ChR2 majority that failed to self-stimulate in terms of their performance on the 2-choice sucrose task, all showed similar ratios of preference for *Sucrose+Laser* over *Sucrose Alone* (8-sec self-stimulator showed a 3:1 preference; non-self-stimulators at almost 4:1 preference) $t_{(3)}$ =0.293, p=.788).

MeA ChR2 does not induce maladaptive attraction for a shock-delivering object in most rats

When given the chance to voluntarily interact with a shock-delivering rod, most MeA ChR2 rats and eYFP rats both made similar amounts of contacts ($F_{1,10}$=0.22, p= 0.65). On the first day, MeA ChR2 rats touched the shock rod 7.6 ± 1.0 compared to 6.5 ± 2.5 touches by eYFP controls. On the second day, both MeA ChR2 and eYFP rats decreased their total number of contacts to 3.5 ± 1.9 by MeA ChR2 and the two eYFP rats made only 2 contacts. During the third test day, MeA ChR2 made 0.8 ± 0.25 contacts while controls each made 1 contact. On the fourth day when no laser was present, MeA ChR2 made the same overall number of contacts 0.8 ± .25 compared to the previous session accompanied with laser (t_9=1.85, p=0.097).

MeA ChR2 and eYFP spent an equal amount of treading throughout the laser-paired test session ($F_{1,10}$=3.1, p=0.11). The largest portion of time spent treading occurred on the first day for a duration of 38.4 ± 37.4 sec by eYFP controls and 14.7 ± 4.1 sec by MeA ChR2. Treading decreased for all groups on the second day down to 4.5 ± 3.5 sec by eYFP controls and 4.7 ± 1.9 sec by MeA ChR2 and on the third day to 0.3 ± 0.25 sec by eYFP group and 6.3 ± 2.6 sec by MeA ChR2. When laser was removed on the final test day, all rats continued to tread at the same level during the first three test days at 1.1 ± 1.1 sec in controls and 6.2 ± 2.9 sec in MeA ChR2.

While as a group MeA ChR2 failed to increase attraction for a painful shock rod, 2/10 rats made over 10 contacts on both the first and second day (1 male and 1 female), and the male rat continued to make over 10 contacts on the third day. With MeA ChR2 present during the first three days this rat received an average of 17.5 ± 1 shocks but did not make any contact to the shock rod in the absence of laser stimulation. This rat was also one of only three MeA ChR2 rats that ever demonstrated chewing behavior directed towards the shock rod, and subsequently went on to directly chew the rod during all three laser-paired sessions for an average of 6.8 ± 4.6 seconds per session. Importantly when laser excitation was removed during a final shock rod

encounter, there were no instances of chewing the shock rod. This indicates that shock rod attraction was not simply being driven by a laser-induced increase in ingestive chewing behavior. For example when scored for time spent chewing on the bedding in the chamber, MeA ChR2 rats spend a total time of 3.2 ± 1.5 sec the first day, 4.6 ± 1.3 sec on the second laser session, 6.7 ± 2.3 sec on the third session. In the absence of photostimulation, MeA ChR2 rats continued to chew the bedding spending 3.9 ± 1.7 sec (Fig. 3.8). When looking at the behavior of the individual rat showing chewing of the shock rod, they appeared to spend less time chewing the bedding on days 1-3 and spent an equal amount of time chewing the bedding as both eYFP controls and MeA ChR2 rats that did not show attraction during the no laser session.

As another measure of attraction, all rats were additionally scored for time spent closely sniffing and hovering over the shock rod. MeA ChR2 rats did not demonstrate more time hovering over/sniffing compared to eYFP controls ($F_{2,20}$=1.3, p=0.30), spending 37.9 ± 7.5 sec on day 1, 15.9 ± 4.8 sec on day 2, 26.2 ± 8.3 on day 3, and 22.7 ± 7.6 sec during the no laser session. Looking at the MeA ChR2 rat that demonstrated the most laser-induced attraction, we observed that they spent almost five times longer investigating the shock rod with laser present averaging 64.1 ± 15.9 seconds compared to only 12.7 seconds when laser was no longer present. In contrast, eYFP controls spent 16.5 ± 4.2 sec sniffing the rod in day 1, 22.6 ± 13.9 sec in day 2, 19.5 ± 4.9 sec in day 3, and 15.3 ± 4.8 sec in the no laser session.

No sex differences exist during shock rod interactions

Given that MeA is sexually dimorphic, sex difference analyses were completed and suggested that there are no differences between the number of contacts made between males and females while receiving laser stimulation ($F_{2,16}$=0.62, p=0.45) or in the absence of laser

($F_{1,9}$=0.25, p=0.63). For example when laser accompanied shock rod encounters, females made 8 ± 1.7 contacts while males made 7.2 ± 0.89 rod contacts on day 1, on day 2 females made 4 ± 3.5 contacts and males made 3 ± 1.9, and finally on day 3 when females made 3 ± 2 contacts and males made 3.9 ± 1.7 contacts. In the absence of laser on the final test day, both males and females continued to make equal contacts touching for females on average 1 ± 0.58 vs males making 0.78 ± 0.22 rod contacts. When looking at time spent nibbling the rod, males and females both displayed equal amount of time chewing when laser present ($F_{2,16}$=0.20, p=0.67) and neither group chewed in the absence of laser. However 2/3 MeA ChR2 rats that chewed the shock rod were male bringing the average time chewing the shock rod to 1 ± 0.72 seconds for males vs females spending only 0.2 ± 0.22 seconds chewing the shock rod.

MeA ChR2 during Pavlovian fear conditioning does not alter freezing to the CS+

To assess whether MeA ChR2 excitation could induce fearful motivation, rats were trained that an auditory CS+ predicted a mild but aversive footshock. Later these rats received 10 presentations of the auditory CS+ (5 of which were accompanied by laser) and percentage of time spent freezing compared to baseline were recorded. Overall both MeA ChR2 and eYFP controls displayed equal degrees of freezing in response to presentations of the auditory CS+, regardless of laser ($F_{4,40}$=0.22, p=0.92; see Fig. 3.9). Looking closer at MeA ChR2, laser activation never altered the percentage of time spent freezing during cue presentations ($F_{4, 36}$=0.60, p=0.66). Additionally, there were no sex differences observed in laser-induced freezing to the CS+ within MeA ChR2 rats ($F_{4, 32}$=0.176, p=0.94). As expected for eYFP control rats, laser stimulation did not impact freezing at any point during cue presentations ($F_{1, 4}$=0.48, p=0.76).

Analysis of baseline behavior across males and females revealed that there may be a trend for females to be more active when placed into the new chamber compared to males, while it was not statistically significant females spent on average only 2.1 ± 1.2 seconds immobile of the first minute while males spent 9.5 ± 5.1 seconds immobile ($F_{1,9}$=2, p=0.195).

Some MeA ChR2 rats strongly avoid a $CS+_{Odor}$ associated with Pavlovian fear conditioning

In a separate test, rats were placed into a two-chamber apparatus where one chamber contained the olfactory CS+ that was present during the three days of Pavlovian fear conditioning. Unfortunately the video for my second eYFP rat was corrupted and unable to be restored so between-subject comparisons cannot be made. Looking at MeA ChR2 rats, 9 of 10 MeA ChR2 rats spent more time on the opposite side of the chamber, averaging 603.1 ± 106.1 seconds exploring the CS+ side of the chamber vs 1201.6 ±105.5 seconds on the opposite side (t_{10}=-2.83, p=0.02). In comparison, the eYFP rat spent 865 seconds with the CS+ odor and 936 seconds.

Although as a group most MeA ChR2 rats spent the larger majority of their time in the opposite chamber, it is likely driven by 3/10 MeA ChR2 (2 females, 1 male MeA ChR2) that very strongly avoided the $CS+_{odor}$ averaging only 131 ± 69.7 seconds vs 1670.3 ± 68.7 seconds on the opposite side. These 3 'Strong $CS+_{Odor}$ Avoider' rats, *Strong Avoiders*, underwent a second test session on a separate day to determine if this was a stable avoidance, and it indeed confirmed that all 3 continued to avoid the $CS+_{Odor}$ side spending only 189.6 ± 51 seconds there compared to 1611.7 ± 62.7 seconds on the opposite side. Although they strongly avoided the $CS+_{Odor}$ they did not display increased levels of freezing when the auditory CS+ was presented compared the MeA ChR2 rats that did not show strong avoidance ($F_{1,8}$=0.37, p=0.56).

Interesting, looking back to the *Strong Avoiders* behaviors during interactions with the shock rod, the same male rat showed the most number of contacts across all three laser-paired days and spent the longest amount of time chewing the rod and 1/2 female MeA ChR2 *Strong Avoiders* showed the most contacts of female rats and was the only female rat to chew the shock rod on any laser-paired session. On average it appeared that the rats later classified as *Strong Avoiders* received more shocks during laser-paired test encounters with the shock rod. For example, on average *Strong Avoiders* received 10.3 ± 1.9 shocks compared to *Non-Avoiders* who received 6.4 ± 0.9 shocks on day one, on day 2 *Strong Avoiders* received 6.7 ± 5.7 shocks vs *Non-Avoiders* 2.1 ± 1.5, on day 3 *Strong Avoiders* received 8.3 ± 4.4 shocks vs *Non-Avoiders* 2 ± 0.9, and finally in the absence of laser *Strong Avoiders* earned 0.67 ± 0.33 shocks compared to 0.86 ± 0.34 earned by *Non-Avoiders*. Of note there were no anatomical differences between optic fiber placements of *Strong Avoiders* and *Non-Avoiders*.

Discussion

This chapter builds upon a growing body of literature that recognizes MeA as a striatal-like structure operating within Swanson's proposed macrosystem framework to drive motivation for rewards. Here pairing MeA ChR2 with one of two identical sucrose reward was able to increase the incentive value of that option so that rats pursue their laser-paired option almost exclusively. This was most strongly observed in the sucrose two-choice task as well as in the separate progressive ratio test where MeA ChR2 rats worked harder, evident by increased breakpoint, to earn their laser-paired sugar pellet.

Consistent with the hypothesis that pairing MeA ChR2 with an external reward increases the incentive motivation of the reward, MeA ChR2 was unable to maintain instrumental levels of responses when sucrose was no longer present in sucrose extinction. This further demonstrates that the presence of the external sucrose reward is necessary to create this narrowly focused motivation. While most of these same rats went on to self-stimulate MeA ChR2 when contacts to an empty spout delivered short 1-second pulses, they did not do so when the spout delivered long 8 second pulses of laser. While it is possible that some levels of self-stimulation contributed to the preference observed during the two-choice sucrose task, both rats that robustly self-stimulated and those that did not, strongly preferred their *Sucrose+Laser* option. Furthermore, while we only observed one rat robustly self-stimulate for 8-second bouts of laser, all MeA ChR2 rats went on to develop a preference for their laser-paired sucrose option.

Observations from this data chapter on pairing MeA ChR2 with a noxious, shock-delivering rod reveal that most rats do not go on to develop a maladaptive attraction to the rod resulting in excessive chewing of the shock rod. However, upon individual analyses it was seen that 2/10 MeA ChR2 rats received at least 10 shocks during each of the first two sessions while investigating the rod were both rats that were classified as *Strong Avoiders* of the $CS+_{odor}$ following fear conditioning and occurred in 1 male and 1 female. The behavior observed in these two rats that MeA ChR2 can be capable of both driving attraction for a noxious shock and avoidance of a CS+odor that predicts a noxious footshock indicates that MeA may also be capable of perating as an affective mode albiet to a lesser extent that observed in the previous chapter with CeA ChR2.

A growing body of literature has identified the power of physiological inputs into MeA and its pallidal recipient, BNST, including odor sensory information, and hunger signals, to orchestrate motivated behaviors (Jennings et al., 2013; Y. Li et al., 2017; Raam & Hong, 2021; Yao et al., 2017). My findings from this chapter identify a novel role of MeA generating motivation for a food reward and suggesting that there may be multiple affective modes as in the case of the MeA ChR2 rats that demonstrated both incentive motivation for the shock-delivering rod but enhanced fearful avoidance of the $CS+_{Odor}$ following Pavlovian fear conditioning. Future studies may want to probe whether food restriction would synergistically enhance motivation when augmented with MeA photostimulation.

Figures

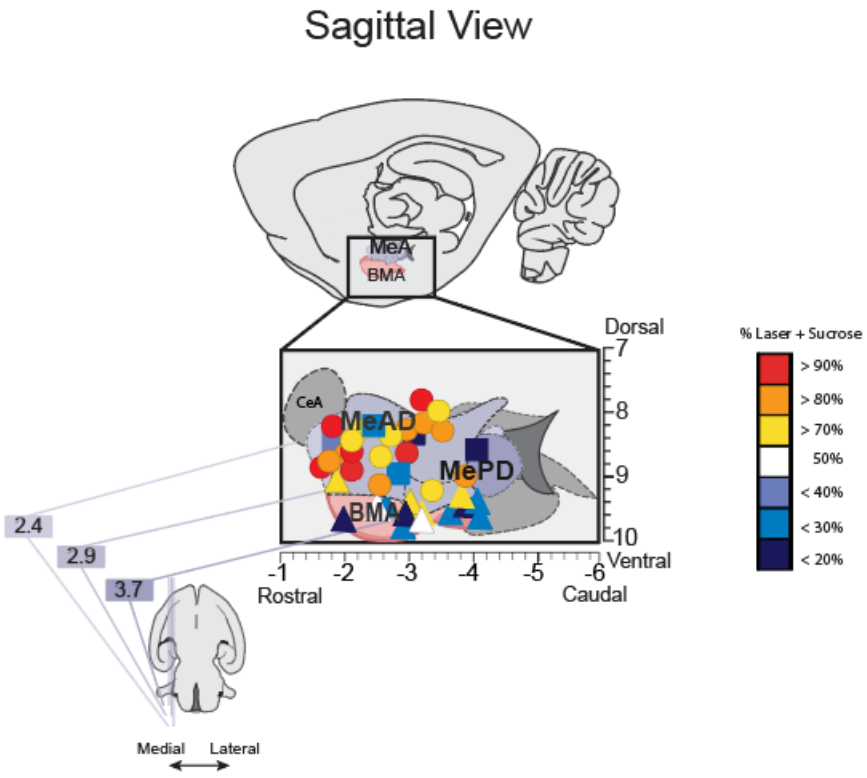

Figure 3.1 Localization of function maps
Function maps of confirmed anatomical placements of virus and optic fibers for MeA ChR2 rats (depicted with circles), BMA ChR2 rats (triangles), and eYFP controls (squares). Color of symbol reflects the percent intensity at which animals responded for the *Sucrose+Laser* option in the two-choice sucrose task. Warmer colors indicate stronger preference, while blues indicate preference for *Sucrose alone*.

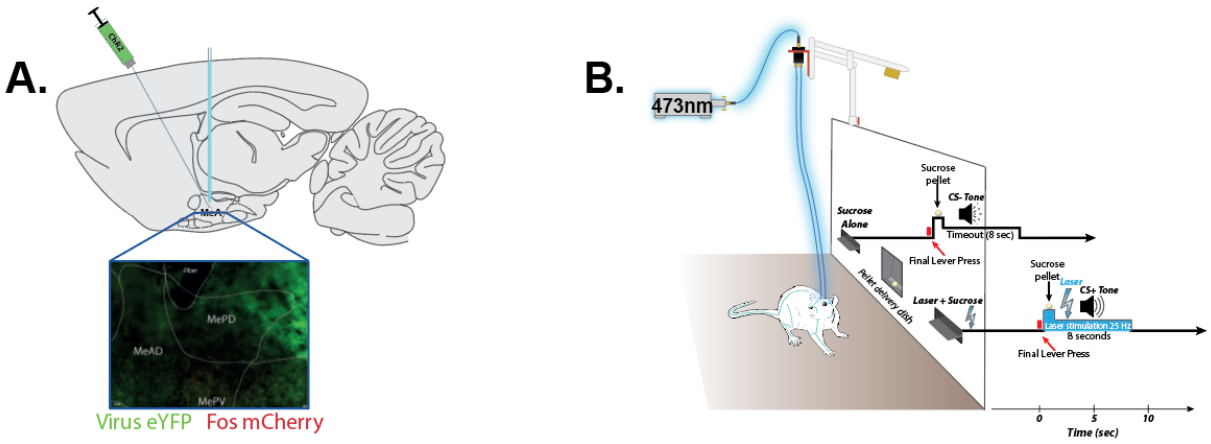

Figure 3.2 Virus expression and two-choice paradigm
A) Photomicrograph (×10 magnification) shows channelrhodopsin virus expression (ChR2; green), and neuronal Fos protein expression (mCherry) immediately surrounding optic fiber tips in the MeA. **B)** Schematic of the two-choice sucrose task adapted from Robinson, Warlow and Berridge, 2014. Successful depressions on the *Laser+Sucrose* lever earned a sugar pellet, 8 seconds of MeA ChR2 (25Hz, 2-3mW), and a paired auditory cue. Presses on the second lever resulted in an identical sugar pellet and 8 seconds of a separate paired auditory cue, without laser.

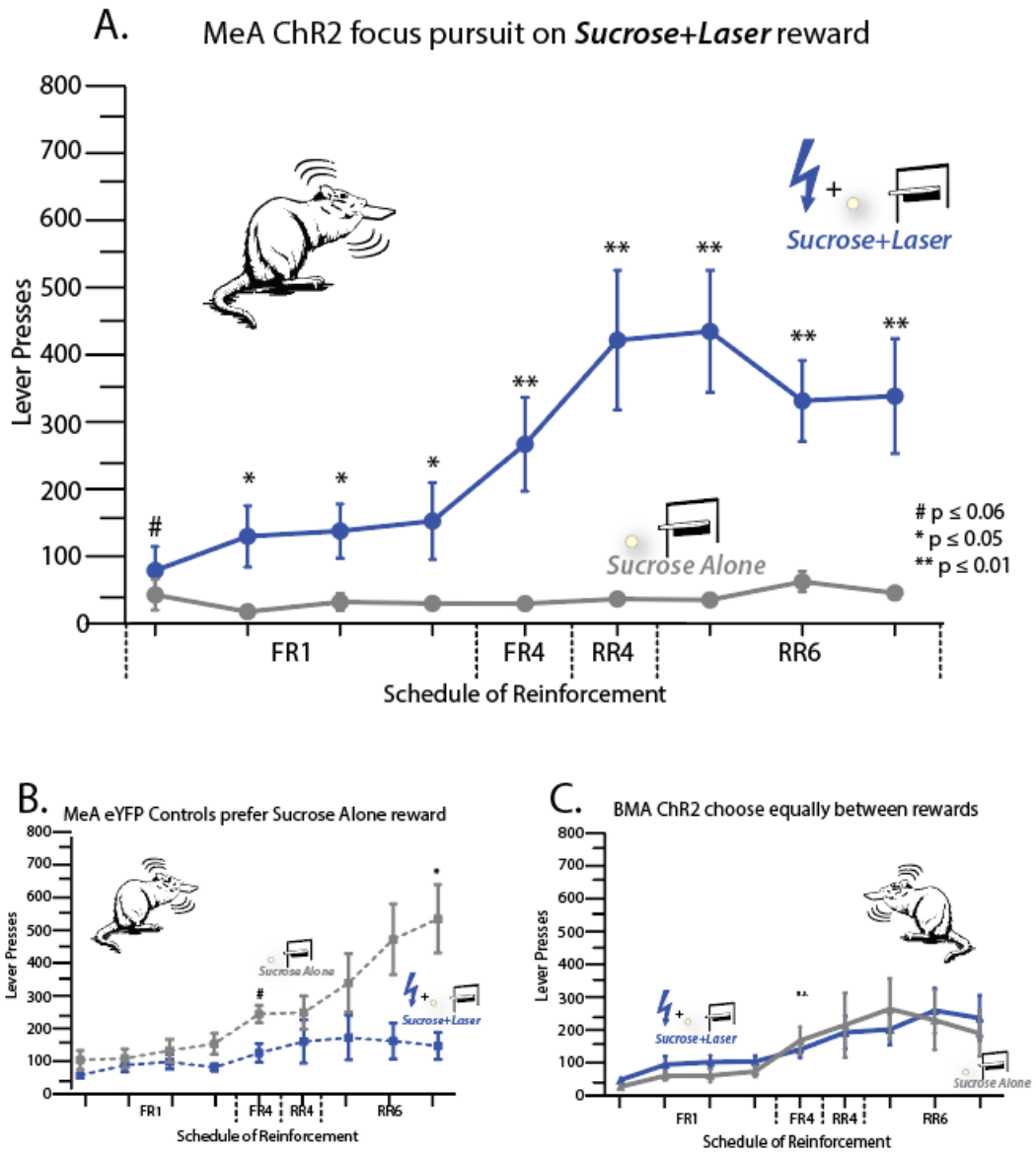

Figure 3.3 MeA ChR2 focuses motivation for a laser-paired sucrose reward
Operant behavior during the two-choice sucrose task is graphed above. **A)** MeA ChR2 (n=15) induces *Sucrse+Laser* preference which begins by day 2 of testing, **B)** Alternatively, eYFP control rats (n=6) eventually establish a preference for the *Sucrose Alone* reward. **C)** Finally, BMA ChR2 rats choose evenly between *Sucrose+Laser and Sucrose Alone* options. n.s. = not significant, **p*<0.05, ***p*<0.01, ****p*<0.001

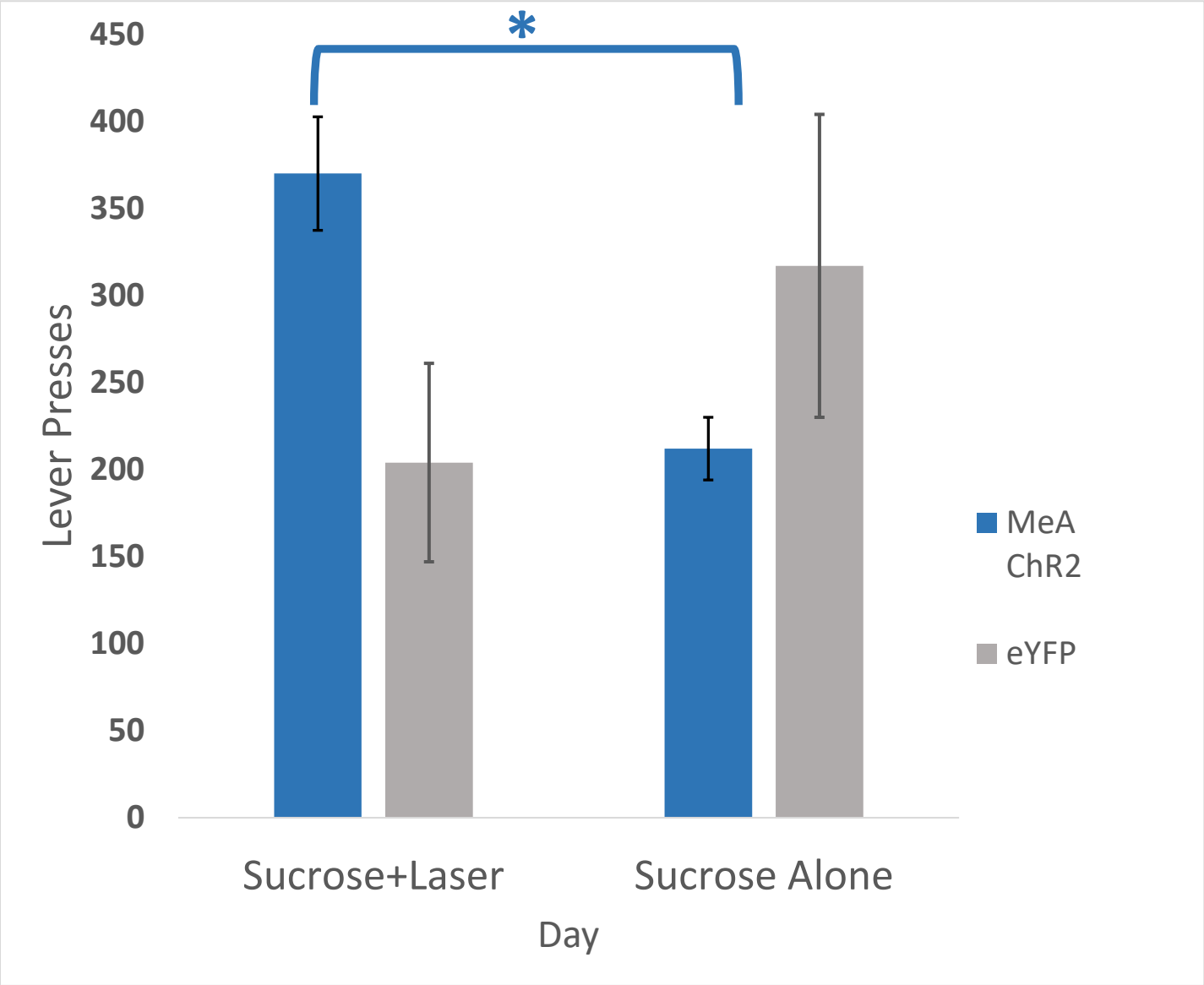

Figure 3.4 MeA ChR2 stimulation enhances breakpoint motivation in progressive ratio test
MeA ChR2 rats (n=10, female n=8, male n=2; blue) pressed more and worked harder to the laser-paired sucrose reward in progressive ratio tests. EYFP controls (n=7, female n=5, male n=2, gray) showed no difference in the number of lever presses between days during progressive ratio. Data shown are means with SEM. *p<0.05

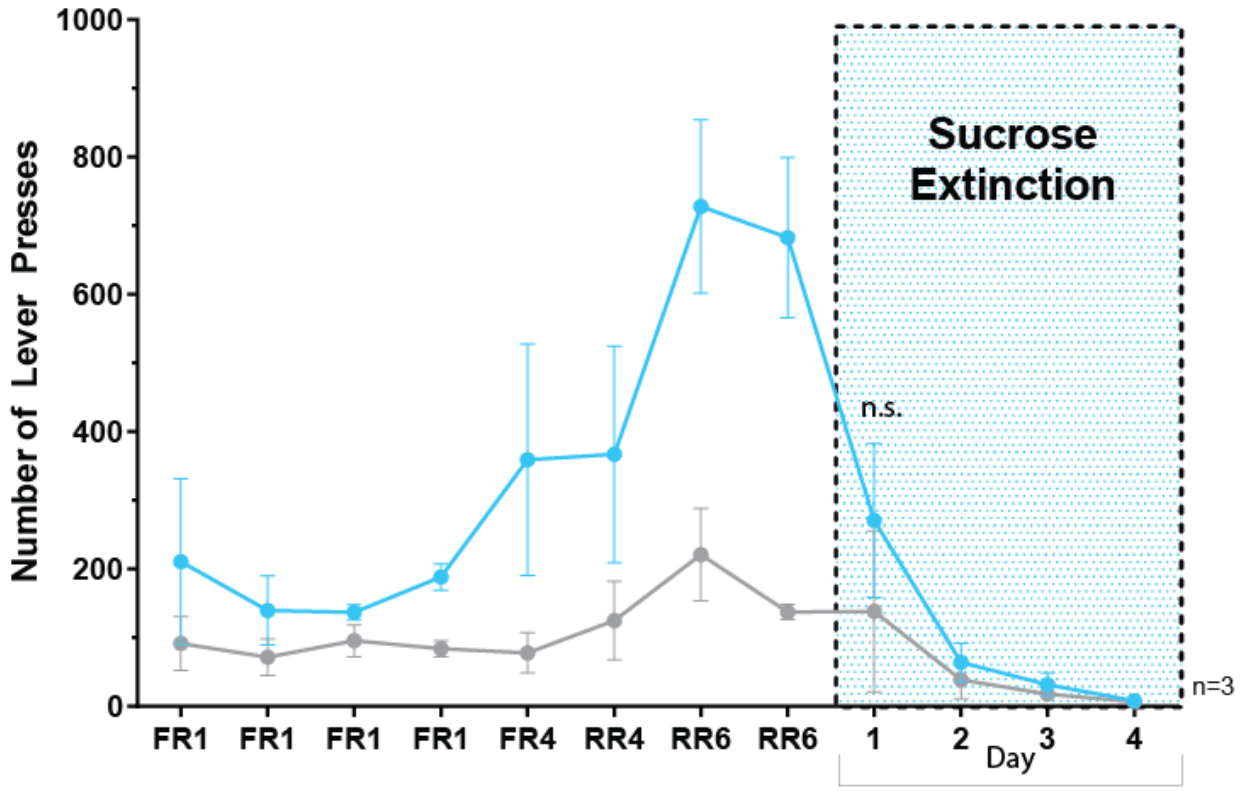

Figure 3.5 MeA ChR2 stimulation alone does not support continued preference in sucrose extinction MeA ChR2 laser (n=3) in the absence of sucrose fails to maintain instrumental responding. Beginning on the first day that sucrose is removed, rats immediately reduced the number of lever presses and began to respond evenly for both options until finally extinguishing responding fully on day 4. Means and SEM reported.

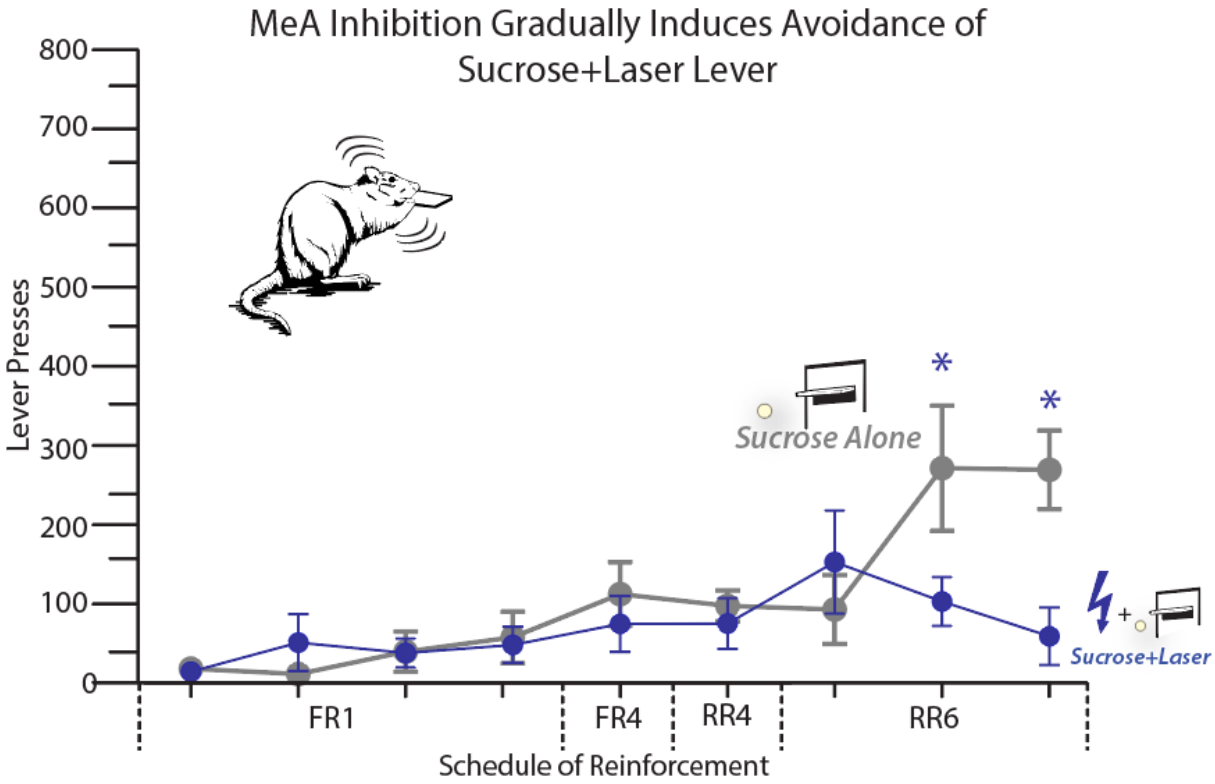

Figure 3.6 MeA inhibition by iC++ gradually induces avoidance of *Sucrose+Laser*. MeA iC++ were tested through the operant two-choice sucrose test and developed a late-emerging avoidance of their *Sucrose+Laser* option by a 4:1 ratio. Means and SEM reported. *$p<0.05$

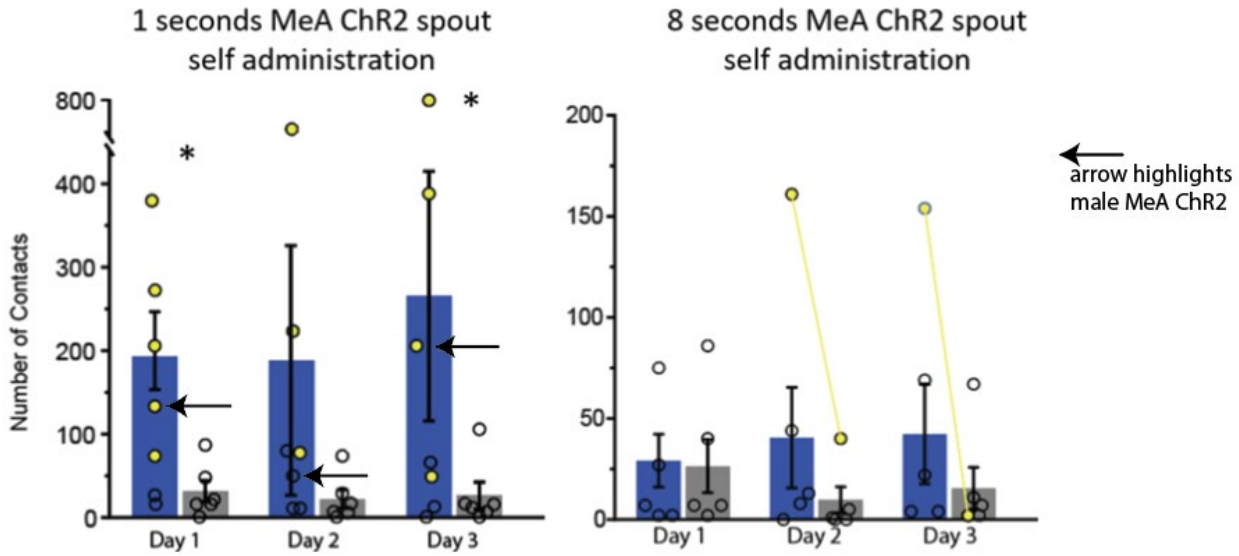

Figure 3.7 Short (1-sec) but not long (8-sec) durations of laser are self-stimulated by most MeA ChR2 MeA ChR2 (robust self-stimulators n=5, females n=4 and males n=1 see arrow for behavior; non-self stimulators n=2) rats meeting criteria for robust laser stimulation (defined as more than 50 laser self-stimulations per 30 minutes session and twice as many contacts to the laser-delivering spout). MeA ChR2 rats self-stimulating for 8-second of laser (robust self-stimulator n=1, non-self stimulators n=4). Rats meeting criteria for robust self-administration are colored yellow. Means and SEM reported. *p<0.05

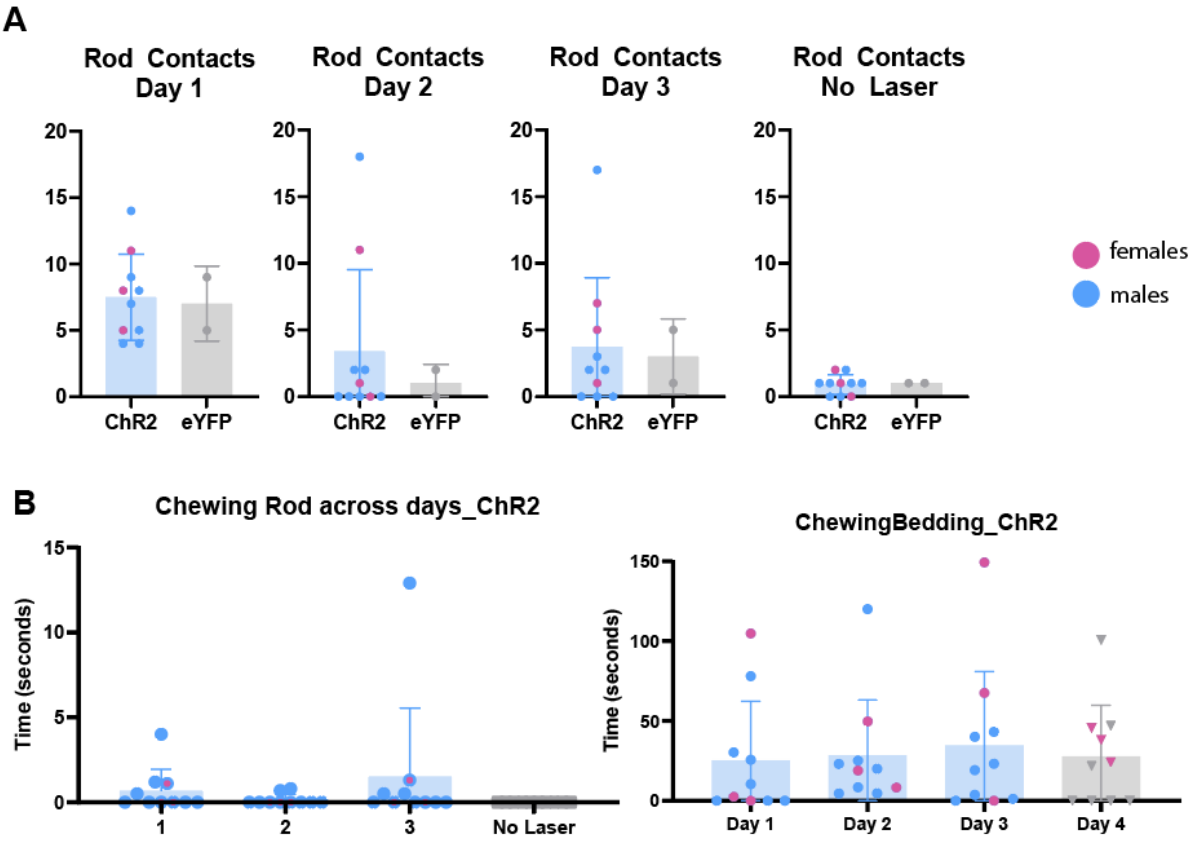

Figure 3.8 MeA ChR2 does not induce maladaptive attraction for a shock-delivering object in most rats **A)** MeA ChR2 (n=10, female=3, male n=7) and eYFP controls (n=2, 1 male and 1 female) both earn similar numbers of shocks across all test days. Individual data points illustrate variation in behavior and highlight the MeA ChR2 male rat that displayed laser-induced attraction to the shock rod. **B)** As a group most MeA ChR2 rats failed to display attraction to the shock rod, the one that made the most contacts went on to spend the longest amount of time spent nibbling on the shock rod. Interesting in **C)** we see that regardless of chewing interactions at the shock rod, many MeA ChR2 rats displayed some chewing of the bedding, however this was not locked to laser. Means ± SEM shown.

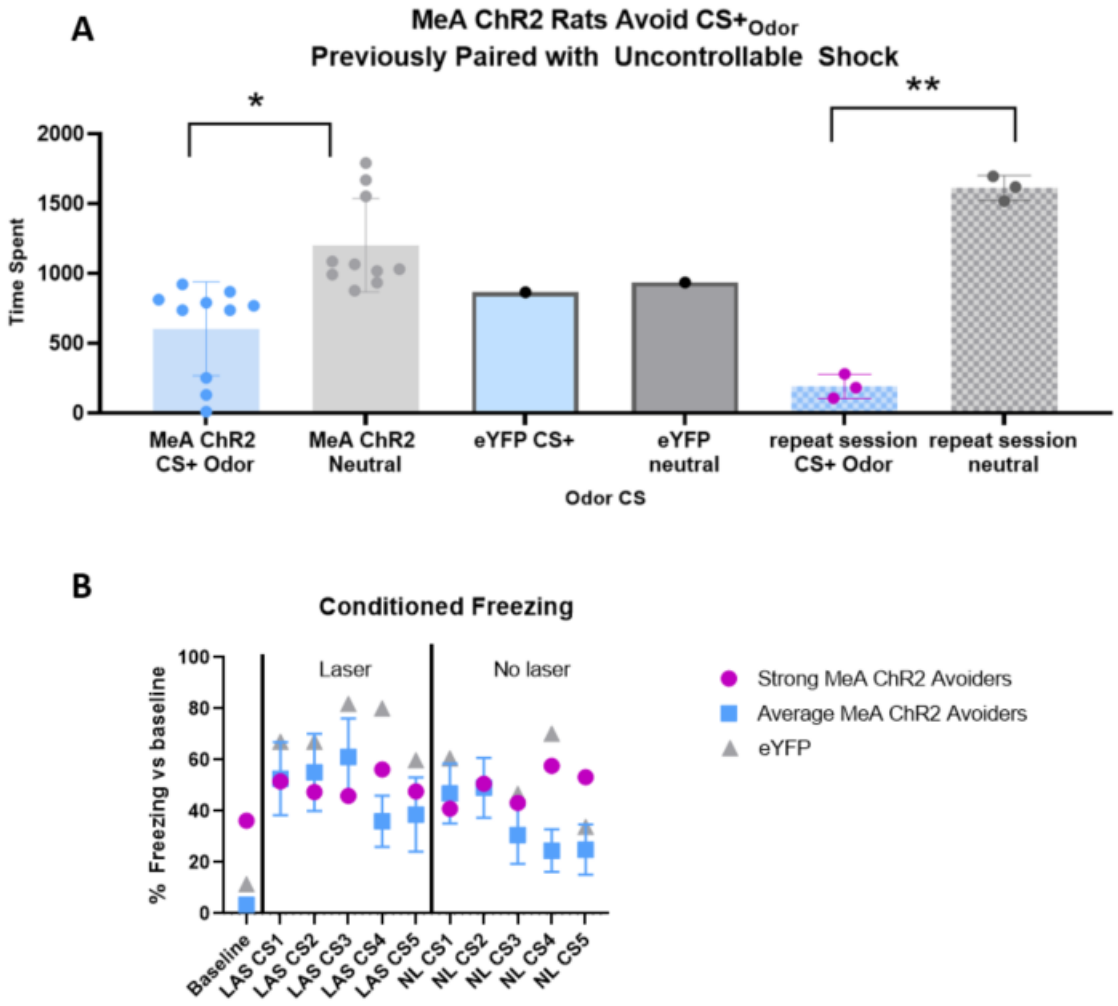

Figure 3.9 MeA ChR2 does not increase freezing to an auditory CS+ that predicted footshock but can amplify avoidance of odor CS+

A) M MeA ChR2 (n=10, female=3, male n=7) all avoided the $CS+_{Odor}$ that was present during Pavlovian fear conditioning. A small group of 3 MeA ChR2 rats demonstrated substantially stronger avoidance of the $CS+_{Odor}$ and underwent a second place test to determine if the avoidance remains stable. In contrast, eYFP (n=2, 1 male and 1 female) spent an even amount of time exploring both sides of the chamber regardless if contained the CS+Odor. **B)** MeA ChR2 rats (strong avoiders of the $CS+_{odor}$ n=3; average avoiders n=7) and eYFP controls (n=2) displayed similar levels of freezing across test days. Strong Avoiders of the $CS+_{Odor}$ displayed a minor increase in baseline freezing during before auditory CS+ were presented. Means± SEM shown.

CHAPTER IV. Medial Amygdala Generates Incentive Motivation to Obtain Intravenous Cocaine and Remifentanil.

Introduction

The involvement of the lateral extended amygdala has long received attention in addiction neuroscience, especially in allosteric-regulated theories and more recently in incentive theories. In contrast, the medial extended amygdala has been largely ignored from these discussions with a few exceptions (Jennings et al., 2013; C. Li & Krashes, 2016; J. C. Nordman et al., 2020; Padilla et al., 2016, 2017). Along with current research that highlight a role of MeA in promoting foraging, aggression, and other motivated behaviors we saw in the previous chapter that MeA ChR2 can create incentive motivation for a food reward. Given that both natural and drug rewards have been shown to recruit dopaminergic mesocorticolimbic circuitry, we next wanted to explore if MeA could focus motivation for an intravenous drug reward (Kelley & Berridge, 2002; Olney et al., 2018; Warlow, Baumgartner, et al., 2020).

Here, I hypothesize that MeA ChR2 can create narrowed pursuit for one of two equal drug rewards (either *Cocaine+Laser* vs *Cocaine Alone* or *Remifentanil+Laser* vs *Remifentanil Alone*) by generating increased incentive value for the MeA ChR2-paired drug. Furthermore, I will demonstrate that MeA can control choice for an intravenous infusion of cocaine paired with MeA ChR2 over an identical cocaine reward without photostimulation. Later when given the

option to work for one of two remifentanil rewards, MeA ChR2 rats eagerly pursued the laser-paired infusion.

Methods

Animals:

Male and female Sprague Dawley rats (male n=6; female n=11) weighing at least 250g at the time of surgery were group-houses and kept in a vivarium with a 12h reverse light/dark cycle, maintained at 21°C. All animals had ad libitum access to water and Purina chow pellets. All experimental procedures describe were approved by the Institutional Animal Care and Use Committee at University of Michigan.

Optogenetic Surgery:

Prior to undergoing surgery, all rats were handled by experimenters for roughly 5 minutes a day for several days to habituate them to human handling. Rats were anesthetized with 5% isoflurane gas and maintained with 1-2% isoflurane throughout the duration of the surgery (Henry Schein, Wixom, MI). All rats received an intraperitoneal injection of atropine (0.04mg/kg) before surgery began to reduce accumulation of fluid in the lungs and during surgeries received subcutaneous injections of isotonic saline (2mL, to prevent dehydration), cefazolin (60mg/kg, to prevent infection), and carprofen (5mg/kg as postsurgical analgesia). Rats were then placed into a stereotaxic device to and received manual bilateral 0.75µLs microinfusions (Hamilton) into the MeA of either the excitatory opsin, AAV-hSyn-ChR2-eYFP (n=15; females n=10, males n=5), or the optically inactive virus, AAV5-hSyn-eYFP (n=5; females n=3, males n=2 however one male subject was lost due to attrition). Virus was manually infused over the course of 8 minutes using a 5µL Hamilton Syringe at a rate of 0.1µL/minute. After allowing 10 minutes for diffusion from the injection site, the microinjector were removed MeA and replaced with bilateral optic fibers (200 µm) implanted 0.3mm dorsal to the injection

site. Rats later received post-operative analgesics 24 hours and 48 hours after surgery and allowed to recover for approximately two weeks before receiving the intra-jugular catheter.

Intravenous Catheter Implantation

After rats had two weeks to recover from the craniotomy, they were again anesthetized at 5% isoflurane and received an intraperitoneal injection of atropine (0.04mg/kg). Subcutaneous carprofen (5mg/kg) and cefazolin (60mg/kg) injections were given at the time of surgery. A small silastic catheter was threaded into the right jugular vein and secured using sutures (inner diameter: 0.28mm; external diameter: 0.61mm; dead volume: 12µLs) as described in Warlow et al., 2017. The opposite end of the catheter attached to the cannula was positioned on the dorsal surface of the back, located just posterior to the shoulder blades. After receiving the intravenous catheter, rats received daily heparinized saline for the duration of the experiment to maintain patency (500IU/mL; Sigma-Aldrich) as well as gentamicin (0.2mL of 5mg/ml) for the first ten days post-op. Rats then received either subcutaneous injections of carprofen or fed bacon-flavored Rimadyl (2mg) at 24hr and 48hr post-op. After a week of recovery, rats were ready to begin self-administration. Prior to beginning self-administration and at the end of testing, all rats received intravenous injections of 0.2ml methohexital sodium to ensure that catheters were patent.

Drug Self-Administration

To determine whether MeA ChR2 activation could focus pursuit of cocaine, rats (MeA ChR2 n=11 (females n=9, males n=2), eYFP n=3 (females n=2, males n=1)) were trained to earn cocaine by nosepoking into portholes located in the operant chamber (Med Associates 30.5 x

24.1 x 21.0 cm). Two portholes were located at the front of the chamber and successful nosepokes always triggered an intravenous infusion of cocaine (50µL over 2.8 seconds, 0.3mg/kg; cocaine salt dissolved in isotonic saline; donated by NIDA) delivered by an automatic pump outside the chamber, followed by a 20 sec timeout in which the noseports retracted and an additional cocaine reward could not be earned. One of these two portholes were assigned the '*Laser+Cocaine'* porthole in which infusions of cocaine were always coupled with 8 seconds of laser stimulation (473nm; 15ms on, 25ms off (25Hz); 2-3mW), while the second active porthole, *Cocaine Alone*, was never accompanied with laser. This designation was counterbalanced across all rats to diminish any effect of noseport preference within the chamber. The two portholes also had designated auditory cues that remained the same throughout the experiment (either tone or white noise; counterbalanced). Each test session lasted for 60 minutes in duration unless they reached 40 infusions in one session, which triggered the end of the test session to prevent overdose. Self-administration days repeated for 10 days, always at a fixed-ratio schedule (FR1).

A separate, small group of rats (MeA ChR2 n=4 (female n=1, male n=3), eYFP=1 male) were instead given the choice to self-administer the short-acting opioid, remifentanil (50µL, 0.002mg/kg) with MeA photostimulation the *Remifentanil+Laser* porthole or an identical dose at the *Remifentanil Alone* porthole (James et al., 1991; Michelsen & Hug, 1996).

Cocaine Extinction

After 10 days of self-administration, a small subset of rats underwent 3 additional days of testing to see if MeA ChR2 stimulation would be capable of maintaining instrumental nosepoking. In these sessions rats were tested in the same operant chambers but now the

Cocaine+Laser noseport resulted in MeA ChR2, the auditory stimulus, but no infusions of cocaine.

Progressive Ratio

To further assess whether MeA ChR2 could increase the incentive motivation to earn intravenous cocaine, some rats (n=2) underwent two days of progressive ratio testing immediately after the 10 days of self-administration that lasted for one hour each. During these two test days rats had access to either their *Laser+Cocaine* porthole followed by *Cocaine Alone* porthole, the order was counterbalanced across rats. The effort required to earn an infusion of cocaine increased with every successful infusion in an exponential progressive ratio schedule PR=[$5e^{(reward\ number*0.2)}$]-5; see (Richardson & Roberts, 1996). As with the self-administration test days, each infusion of cocaine resulted in a 20 sec timeout period. Rats that failed to earn at least two infusions were excluded from analysis.

Histology

After the completion of testing, rats were deeply anesthetized with sodium pentobarbital (0.8mL) and transcardially perfused. Brains were extracted and post-fixed in a 4% paraformaldehyde solution for two days at 4°C before being transferred to a 25% sucrose solution and stored at 4°C. Brains were then sectioned into 40 µm sections using a cryostat (Leica) and stored in cryoprotectant until ready to be processed. Next sections were rinsed in 0.1M sodium phosphate buffer three separate times, each rinse for 10 minutes. After they were rinsed, sections were mounted onto slides and later cover slipped. Once mounted to slides, a fluorescent microscope (Leica) was used to confirm virus expression and fiber placement were

localized MeA. The confirmed sites of the optic fiber were then transferred onto coronal sections from Paxinos and Watson (2007).

For longer description of methodology please see, Warlow et al., 2017.

Statistical Analysis

Data was analyzed utilizing the software SPSS to run repeated-measure ANOVAs and t-tests with Bonferroni corrections. For non-normally distributed data including all progressive ratio tests, Friedman's two-way ANOVAs were used as nonparametric within-subject tests and Kruskal-Wallis one-way ANOVAs for between subject tests, followed by Wilcoxon sign-ranked/ Mann-Whitney tests for individual comparisons. Effect sizes for parametric tests were calculated using Cohen's d and for nonparametric tests using $r = \frac{Z}{\sqrt{N_1+N_2}}$. For all analyses, the significance level was set at $p < 0.05$, two-tailed.

Results

MeA ChR2 pursue laser-paired intravenous infusion of cocaine

To examine whether MeA ChR2 would intensify and focus pursuit of an intravenous cocaine reward in the same manner as the sucrose reward, rats could choose to MeA stimulation paired with intravenous cocaine from one noseport or choose an identical dose of intravenous cocaine from a separate noseport. MeA ChR2 rats received more cocaine infusions coupled with laser stimulation ($F_{1,10}$=5.34, p=0.043; see Fig 4.1).

Beginning on day 1, MeA ChR2 made 3.8 ± 1.3 *Cocaine+Laser* nosepokes and 5.1 ± 1.9 *Cocaine Alone* responses (t_{10}=-.124, p=0.90; see Fig. 4.2). By the 8th day of testing, MeA ChR2 rats began to earn a larger proportion of their cocaine infusions from the *Cocaine+Laser* porthole, earning on average 6.6 ± 1.8 compared to only 1.8 ± 0.5 *Cocaine Alone* (t_8=3.4, p=0.010). This preference remained on the last day of testing with MeA ChR2 responding 6.1 ± 2.1 for *Cocaine+Laser* and 2 ± 0.7 *Cocaine Alone* responses (t_8=2.7, p=0.03; see Fig 4.2, 4.4). Although MeA ChR2 focused pursuit for the *Cocaine+Laser* reward, they continued to earn a stable amount of total cocaine infusions across all 9 test days ($F_{1,8}$=2.39, p=0.16) and never differed from eYFP controls in escalation ($F_{8,80}$=0.32, p=0.96).

In contrast, to the 3:1 preference for *Cocaine+Laser* MeA ChR2 rats developed ($F_{1,8}$=7.87, p=0.02), eYFP control rats never discriminated between the two nosepokes and earned equal numbers of intravenous infusions from both the *Cocaine+Laser* and *Cocaine Alone* portholes ($F_{1,2}$=0.296, p=0.47). Similar to MeA ChR2 rats, eYFP controls failed to escalate cocaine intake across days ($F_{1,2}$=0.36, p=0.87).

This study did not identify any sex differences in laser-induced preference for MeA ChR2 paired cocaine during self-administration ($F_{1,9}$=0.33, p= 0.597), however it is of note that

this sample size included a larger percentage of female (n=9) compared to male (n=2) MeA ChR2 rats. Given published sex differences in the acquisition and administration of cocaine self-administration, the total number of cocaine infusions each day were summed and compared across both males and females (M. Hu & Becker, 2008; M. Hu, Crombag, Robinson, & Becker, 2004; Jackson, Robinson, & Becker, 2006; Swalve, Smethells, & Carroll, 2016). While there was a lot of variability in the total number of cocaine infusions across days, females appeared to intake more infusions than males. For example, we saw that on day 1 males earned 4.5 ± 3.5 infusions compared to 8.2 ± 2.5 infusions earned by the females and continued to reflect this slight increased drug-taking in the final test session in which males earned 3 ± 1 cocaine infusions whereas females earned 7 ± 2.3 total infusions.

MeA ChR2 pursue laser-paired intravenous infusion of remifentanil

MeA ChR2 rats given the option to respond for *Remifentanil+Laser* vs *Remifentanil Alone* showed a trend for preferring the laser-paired reward ($F_{1,3}$=9.4, p=0.055; see Fig. 4.3). On the first day they made 10.3 ± 5.3 nosepokes for *Remifentanill+Laser* vs only 3 ± 1.2 *Remifentanil Alone*, on day two they increased to 19.3 ± 6.7 laser-paired responses compared to 3.8 ± 0.95, on day three they earned 18.3 ± 7.2 laser-paired infusions vs 2 ± 1.1, on day four they received 21.8 ± 9 laser-paired vs 3.2 ± 3.3, and on day five earning 26.5 ± 8.9 compared to 2 ± 1.3 *Remifentanil Alone* infusions. There was a steep decrease in responding on the next two test sessions that prompted a catheter patency check. It was at this point two of the four MeA ChR2rats had malfunctioning catheters and failed to become ataxic within 10 seconds following a brevital check. These rats were unable to complete testing but the remaining two MeA ChR2 rats continued. The remaining two rats with patent catheters went on to make 8 ± 0

Remifentanil+Laser vs 1 ± 1 *Remifentanil Alone* on day 6, on day 7 4.5 ± 1.5 laser-paired and 2 ± 1 *Remifentanil Alone,* on day 8 9.5 ± 4.5 compared to 5 ± 2 nonlaser infusions, on day 9 23 ± 15 compared to 2 ± 0, and on day 10 26 ± 15 *Remifentanil+Laser* vs 3 ± 0 *Remifentanil Alone.* The eYFP rat initially chose evenly between the laser and nonlaser paired reward but began to demonstrate a preference for the *Remifentanil Alone* reward by the 6th day of testing that persisted across the remaining test sessions.

Discussion

Here we find that pairing MeA ChR2 with an intravenous drug reward is capable of inducing a preference for the laser-paired reward. Importantly, when a pair of rats underwent cocaine extinction in which successful porthole responses no longer earned intravenous cocaine but continued to earn MeA ChR2, laser by itself was not enough to maintain responding. This suggests that like in the previous chapter where MeA ChR2 induced a strong *Sucrose+Laser*, this laser by itself (especially at longer, 8-second durations) are unable to maintain this preference without an external reward.

MeA ChR2 rats but not eYFP controls gradually developed a preference for the *Cocaine+Laser* option by the 8th day of testing. This preference was not capable of increasing motivation to earn their *Cocaine+Laser* when tested for breakpoint in a progressive ratio task. This failure to increase breakpoint is in contrast to our hypothesis and previous studies from the lab demonstrating that CeA ChR2 creates both focused pursuit and increased motivation for laser-paired intravenous cocaine.

While sample sizes are fairly small to effectively power a sex difference analysis, here we observed that while both males and females demonstrated preferences for MeA ChR2-paired intravenous cocaine, females may be earning more total infusions. This observed difference may reflect sex differences in the effects of psychomotor sensitization that cocaine has, in which females show enhanced locomotion to cocaine. While this study did not directly assess psychomotor sensitization, future studies may want to examine sex differences in MeA ChR2-induced incentive motivation for drug rewards. Furthermore, previous literature has indicated

that there are differences in the acquisition of drug taking dependent upon circulating gonadal hormones in which females more readily acquire cocaine self-administration compared to males. Here we did not record and track estrus cycle in the female rats but these results together with previous literature suggests that it would be worthwhile to explore whether this slightly enhanced drug consumption in female MeA ChR2 rats is related to laser-induced sex differences or baseline sex differences in self-administration.

When a small, separate group of rats had the opportunity to self-administer *Remifentanil+Laser* there appeared to be a preference for the laser-paired infusion but significance could not be detected. This is possibly due to the small sample size reported here or like MeA *Cocaine+Laser* it may take longer to reach acquisition of self-administration. Of interest to note, the group that underwent remifentanil self-administration were predominantly male versus our predominantly female group in cocaine self-administration. It is possible that this lack of significance may be partially explained by the slower acquisition of self-administration in male rats (Thorpe, Lacy, & Strickland, 2020). Perhaps if future studies were to investigate female rats, we would observe a similar late-emerging preference for the MeA ChR2 paired intravenous remifentanil as observed with intravenous cocaine.

Interestingly, MeA ChR2 rats as a whole responded and earned more infusions of remifentanil compared to cocaine during self-administration. While it is appreciated that cocaine and remifentanil have differences in their effect on neurotransmitter release, it may be the case that rats more readily acquired self-administration for the short-acting opioid.

Figures

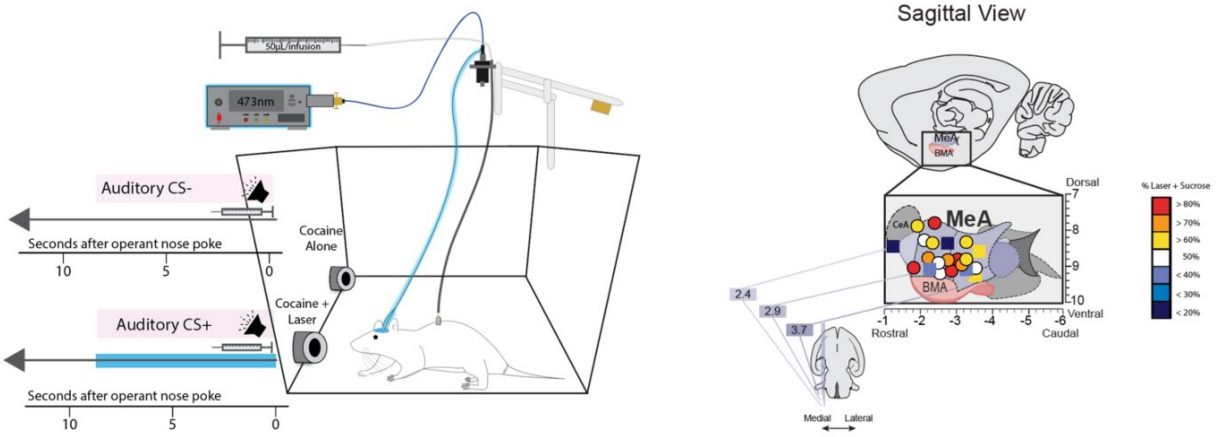

Figure 4.1 Schematic of MeA ChR2 drug self-administration and confirmed neuroanatomical placements

To the left there is an overview of the experimental setup for drug self-administration. On the right side are confirmed anatomical placements for MeA ChR2 rats (n=8; each circle represents one fiber placement so that each rat has 2 with the exception of one rat that lost a fiber during testing) and eYFP controls (n=3; each fiber placement represented by a square). Colors signify the strength of the *Cocaine+Laser* preference during the last 3 test days.

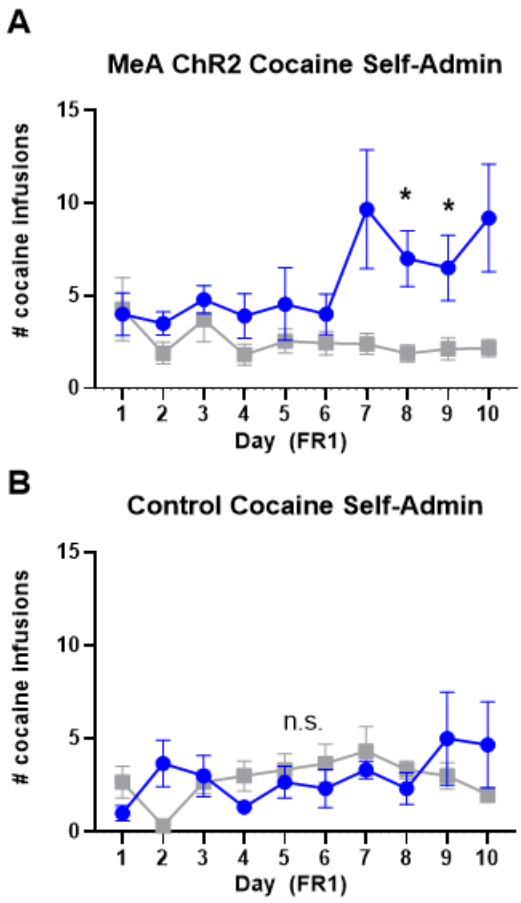

Figure 4.2 MeA ChR2 rats develop a late-emerging preference for *Cocaine+Laser* while eYFP controls choose evenly.
A) MeA ChR2 rats (n=11; males n=5, females n=6) began preferring their *Cocaine+Laser* reward by day 8 and 9 of testing. While **B)** eYFP control rats choose evenly between both noseports throughout the 10 days of self-administration. Means±SEM shown. *p<0.05

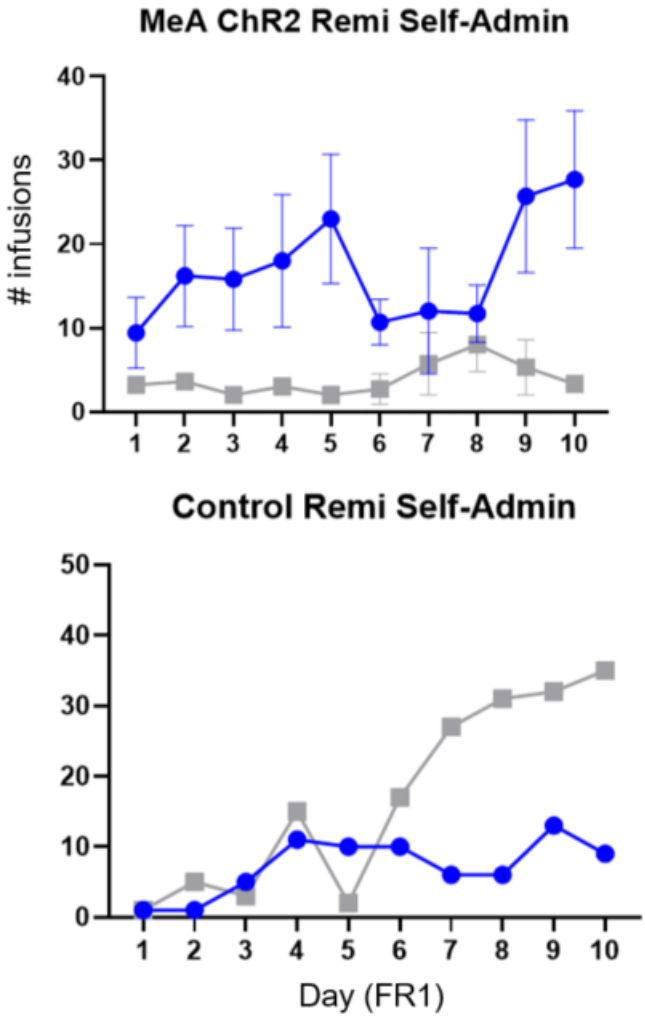

Figure 4.3 MeA ChR2 focuses motivation for laser-paired infusion of remifentanil
MeA ChR2 (n=4; 2 males and 2 females) were able to successfully complete self-administration days 1 through 5. Starting day 6, 2 MeA ChR2 females were excluded due to lack of catheter patency, so test days 6-10 dropped down to two subjects. Together we see a trend for MeA ChR2 rats to prefer their *Remifentanil+Laser* over the *Remifentanil Alone.* This is in contrast to the lower graph which displays behavior from one eYFP control rat. Means and SEM reported for MeA ChR2 and data from single eYFP control below. Opposite to the observed laser preference in MeA ChR2, eYFP controls eventually developed a preference for the *Remifentanil Alone* reward by day 6.

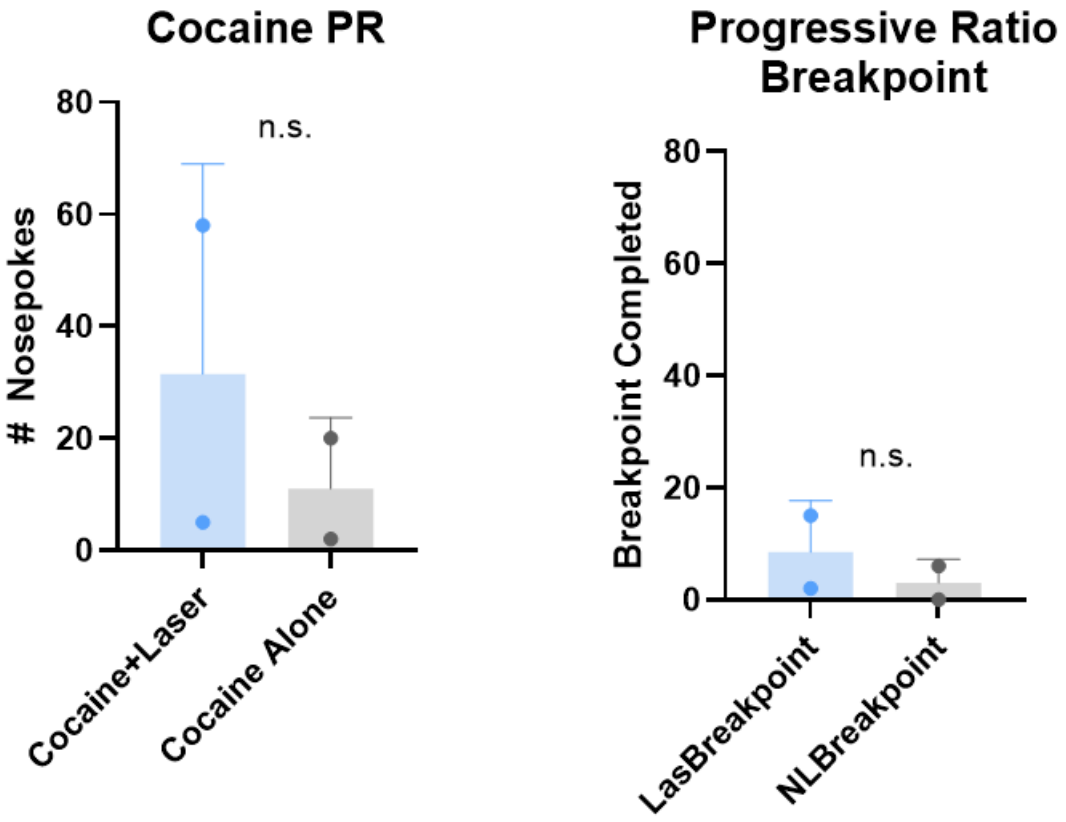

Figure 4.4 MeA ChR2 does not amplify motivation to earn intravenous cocaine
MeA ChR2 rats (n=2) underwent a progressive ratio to measure motivation to consume cocaine. Here we see large variation in motivation to earn cocaine. Means and SEM are reported for number of nosepokes on the left and the breakpoint completed on the right. n.s. = not significant

CHAPTER V. General Discussion

Synopsis

Affective neuroscience has steadily been revealing how the brain is capable of monitoring physiological needs, cognitive desires, prior experience, and integrating it all into a motivated behavior that is flexible and reflects situational demands. Here I demonstrated how positive, incentive motivation can be powerfully generated by both the central amygdala (CeA) and medial amygdala (MeA) to direct motivation towards one of two available sugar or drug rewards. CeA ChR2 was both capable of creating incentive motivation for sugar, cocaine or a noxious but controllable shock-delivering rod in addition to creating fearful avoidance of a similarly noxious but uncontrollable footshock. In contrast, only two individual MeA ChR2 subjects tested were able to flexibly shift from incentive motivation during shock rod encounters to fearful avoidance in conditioned place preference.

In Chapter II, my colleagues and I presented novel findings of directing motivation for a competing cocaine reward or a sucrose reward when one of the two are paired with CeA ChR2. In a separate experiment CeA ChR2 stimulation could generate maladaptive attraction for a noxious shock that is sought out, that animals will work for associated cues, and that they will voluntarily investigate, bite and nibble a rod that delivers electric shock upon contact.

Interesting, when CeA ChR2 was coupled with a CS+ that predicted noxious shock delivered uncontrollably via footshock was capable of inducing increased fear expression and avoidance.

In Chapter III we observed that while MeA was capable of generating extreme incentive motivation to obtain a sugar reward. MeA ChR2 was capable of supporting self-stimulation when administered in short, 1 second pulses but not longer, 8 second pulses and is consistent with previous studies examining intracranial self-stimulation in the amygdala. Unlike in CeA, MeA ChR2 was unable to generate maladaptive attraction for the shock rod in most animals, there was one case of a MeA ChR2 rat who consistently showed laser-induced attraction and nibbling towards the shock rod. This same rat went on to strongly avoid the $CS+_{Odor}$ that was previously paired with an uncontrollable footshock.

And lastly Chapter IV identifies a novel finding that MeA can focus motivation for drug rewards including intravenous cocaine and remifentanil. Together this dissertation highlights the eloquent neuroanatomical studies that identify CeA and MeA as striatal-like and demonstrate how these striatal-like amygdala regions can focus incentive motivation for sugar, intravenous cocaine, a shock-delivering object, and elicit self-stimulation at low stimulation durations.

Powerful Affective Mode in CeA, Possible Affective Mode in MeA

In these studies, CeA was observed to powerfully able to flip from incentive to aversive motivation than MeA. This indicates that while both MeA and CeA possess the capacity to flip between affective modes, CeA may be better suited to orchestrate the flip from a negative shock to a positive incentive. Here distant c-fos activation following MeA ChR2 stimulation-paired

interactions with sucrose and intravenous drugs were not quantified but this would be a great next step in identifying whether like the CeA, the MeA also recruits mesocorticolimbic circuitry to drive this focused pursuit of rewards. While both are striatal-like structures, there are considerable differences in connectivity with up and downstream structures. It may be these specific pathways that are driving the observable differences seen with cocaine self-administration and maladaptive shock rod attraction.

One alternative possibility is that the behavioral tests run here were not the best suited to capture different affective modes in MeA. Reflecting on the published literature, studies of MeA orchestrating sociosexual behavior support the idea that MeA can flip between positive-valenced behaviors such as prosocial and parental care and negative-valenced behaviors such as aggression and infanticide depending upon prior experience.

Along with this idea, studies investigating CeA-elicited motivated behaviors include predatory behavior and perception of noxious stimuli (Han et al., 2017). For instance, one substantial difference between CeA and MeA that could be in the recruitment of downstream structures. In chapter II, we saw that CeA ChR2 rats demonstrated attraction for the shock rod in terms of time spent near the rod, number of contacts, and time spent chewing on the shock rod. Notable key efferents of CeA are the periaqueductal gray (PAG) and reticular formation which have been implicated in their roles of predatory hunting and consumption of prey, respectively. More specifically, this paper found that selective activation of CeA to GABAergic neurons within the parvocellular reticular formation was capable of inducing lethal attacks of prey, artificial prey, inanimate objects, and even ‘fictitious eating’ (mouth movements in the absence

of food or external stimuli). In these studies we targeted all neurons within CeA (chapter II) or MeA (Chapter III-IV) and were not selective for specific projections or cell types. While it may be possible that some laser-induced chewing towards the shock rod may be due to activation of this pathway, results from the 'dummy rod' and wooden block encounters revealed that stimulation of these hSyn neurons in CeA are not sufficient at driving increased time spent chewing neutral objects. Perhaps while the shock rod and Pavlovian fear conditioning behavioral assays were able to capture this flexible shift in affective modes, future studies may try to capture this flexible shift in MeA by investigating MeA ChR2 stimulation in different paradigms.

In Chapter IV we observed another distinct instance in which CeA and MeA may differ. One possibility for this difference may be the efferent projections exiting CeA and MeA may differ critically for drug self administration tasks but not operant tasks related to food intake (C. K. Funk, O'Dell, Crawford, & Koob, 2006; Haight, Fuller, Fraser, & Flagel, 2017; Koob & Schulkin, 2019; Koob & Volkow, 2010; Koob, 2010; Koob et al., 2014; Specio et al., 2008; Zorrilla et al., 2014). For instance, the Allosteric Model of drug-taking ascribes a lot of the CeA-mediated drug taking behavior to a population of corticotropin releasing hormones (CRH) within the lateral extended amygdala. While there are populations containing CRF-neurons in MeA, it is much smaller in comparison to the population described in CeA (Dabrowska, Hazra, Guo, Dewitt, & Rainnie, 2013; Jiang, Rajamanickam, & Justice, 2019; Koob & Schulkin, 2019; Merchenthaler, 1984; Pomrenze et al., 2015; L W Swanson, Sawchenko, Rivier, & Vale, 1983). Another alternative possibility is that there are baseline sex differences across the CeA and MeA ChR2 experiments. While the previous study that demonstrated CeA ChR2 can increase

incentive motivation for an intravenous cocaine reward, all of the animals tested in that experiment were female rats while in the present study we had both male and female rats.

MeA ChR2 increases motivation for sucrose but not cocaine

MeA ChR2 rats did not work harder for their laser-paired cocaine reward. This is in contrast to the increased breakpoint MeA ChR2 rats displayed when responding for sucrose in a progressive ratio task, as well as previous reports of CeA ChR2 stimulation increasing motivation for cocaine. MeA rats overall seem to self-administer less cocaine than observed in CeA ChR2 rats, so it is conceivable that a larger number of rewards must be earned to amplify this motivation to administer cocaine. Since MeA did increase effort to work for a sucrose-paired reward, it would be interesting to try and replicate these findings with rats responding at high rates for cocaine or remifentanil. Unfortunately the group that responded at high rates for intravenous MeA ChR2-paired remifentanil were unable to complete progressive ratio due to interruptions in research from the Covid-19 pandemic.

One alternative possibility that could be driving noticeable differences between the two studies is that MeA ChR2 rats that lever pressed for sucrose were gradually trained on increasing schedules of reinforcement (four days of FR1, one day of FR4, one day of RR4, and then 3 days of RR6) over test days before undergoing the progressive ratio test. Future studies should try to gradually increase the schedule of reinforcement to see if this would alter results observed in progressive ratio.

Finally, it is possible that given the short 2 day timeframe of progressive ratio compared to almost 2 weeks of self-administration, the estrus cycle more largely influenced motivation to work for cocaine (Becker & Hu, 2008; Becker, 2016). Since female cycles were not monitored during these studies, future studies should observe and track the estrus cycle to correlate with differences in motivation. However, both males and females demonstrated equal increases in motivation to work for the sucrose reward during the two day progressive ratio test in Chapter III.

Neuroanatomical considerations

While the present experiments used a general optogenetic promoter that transfected all neurons regardless of cell-type, utilizing recently advanced technology and transgenic rat lines may better help to uncover specific neural mechanisms responsible for the observed flip in affective modes (Hagihara et al., 2021). It may also reveal further evidence for direct and indirect pathways from the striatal-like CeA and MeA.

Specifically looking at CeA, D1r neurons are thought to be primarily localized to somatostatin neurons that elicit positive valence and also express prodynorphin; in contrast D2r receptors are predominantly localized to PKC-d (J. Kim et al., 2017). However, there is still debate as to whether these two pathways are functionally distinct when it comes to reward, for instance pilot studies from the Berridge Lab have indicated that rats will self-stimulate CeA D2r (Abtahi, Rodberg, and Berridge, 2019).

Recent evidence has identified two distinct neuron populations in MeA categorized as either D1r neurons that co-release GABA at downstream terminals in the BNST or MeA D1r neurons that co-release glutamatergic neurotransmitters in the ventromedial hypothalamus (VMH). These two discrete populations were observed to have opposing behavioral effects, such that activation of MeA projections that released GABA in the BNST were shown to promote approach/exploratory behavior when exposed to a predator odor, a robobug, or tested in a resident intruder paradigm. Oppositely, activation of MeA projections to the VMH were shown to oppose and bias animals towards avoidance/aggressive behavior in these same behavioral tests (Miller et al., 2019). Future studies will help to identify if these direct and indirect pathways maintain this approach and avoidance behaviors, respectively.

Implications for human mental health disorders

Reflecting upon the possible implications for human mental health disorders, these studies help support the notion that substance use disorders are not simply disorders of inappropriate pleasure-seeking. Here we observed that rats could be made to maladaptively 'want' something that is known to be painful and expected to be painful. Such may be the case in severe substance use disorders where individuals consciously report wishes to abstain from taking drugs but feels compelled to continue drug taking behavior. In this study we artificially stimulated neurons within CeA to induce the 'wanting for what hurts', although imaging studies from human literature has revealed that there may be different biophysical signatures in amygdala activity at baseline which may reflect susceptibility or presence of mental illness (Bas-

Hoogendam, van Steenbergen, van der Wee, & Westenberg, 2020; Donegan et al., 2003; Hariri et al., 2005; Joos et al., 2011; Matos et al., 2020).

Reflecting upon the results from Chapters III and IV, it is of special interest to investigate how alterations may be occurring in MeA to influence susceptibility or resiliency to mental health illnesses. Given that MeA is a sexually dimorphic structure that is impacted by cycling gonadal hormones at points across the lifespan, it may be especially important to investigate contributions of MeA functionality in humans. Here sexually naive females appeared to self-administer more intravenous cocaine in comparison to sexually naïve males. This supports previous animal and human studies that indicate an increased propensity to acquire and escalate self-administration of drugs (Becker & Hu, 2008; M. Hu & Becker, 2008; M. Hu et al., 2004; Jackson et al., 2006; Moran-Santa Maria, Flanagan, & Brady, 2014; Terner & de Wit, 2006). Importantly, these results highlight a role of MeA in incentive motivation for food and drug rewards and suggest that future research in humans may want to look at MeA for a target of pharmaceutical interventions for substance use and addictive-related disorders.

This work supports the idea that the amygdala can detect and augment the saliency of cues that already possess some valence, such as the case where CeA ChR2 rats would repeatedly endure shocks to investigate the shock rod but these same rats would not investigate a 'dummy rod' with identical same laser stimulation parameters. In the case of MeA ChR2-paired sucrose, when sucrose was no longer present these rats immediately dropped off their responding rates and did not continue to self-stimulate the 8-second laser. Finally, this notion was also demonstrated in the rats that received MeA ChR2-paired infusions of cocaine, as once cocaine

was removed they ceased responding at the noseports. Together, these experiments support the anatomical framework of the telencephalon laid out by Swanson and colleagues and behaviorally demonstrate that CeA and MeA are functionally operating as striatal-like structures within the extended amygdala.

References

Adekunbi, D. A., Li, X. F., Lass, G., Shetty, K., Adegoke, O. A., Yeo, S. H., … O'Byrne, K. T. (2018). Kisspeptin neurones in the posterodorsal medial amygdala modulate sexual partner preference and anxiety in male mice. *Journal of Neuroendocrinology*, *30*(3), e12572. https://doi.org/10.1111/jne.12572

Ahmed, S. H. (2010). Validation crisis in animal models of drug addiction: beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews*, *35*(2), 172–184. https://doi.org/10.1016/j.neubiorev.2010.04.005

Alheid, G F, & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience*, *27*(1), 1–39. https://doi.org/10.1016/0306-4522(88)90217-5

ALHEID, G. F. (2006). Extended Amygdala and Basal Forebrain. *Annals of the New York Academy of Sciences*, *985*(1), 185–205. https://doi.org/10.1111/j.1749-6632.2003.tb07082.x

Alheid, George F. (2003). Extended amygdala and basal forebrain. *Annals of the New York Academy of Sciences*, *985*, 185–205. https://doi.org/10.1111/j.1749-6632.2003.tb07082.x

Alheid, George F., Beltramino, C., Braun, A., Miselis, R. R., François, C., & de Olmos, J. (1994). Transition Areas of the Striatopallidal System with the Extended Amygdala in the Rat and Primate: Observations from Histochemistry and Experiments with Mono- and Transsynaptic Tracer. In G. Percheron, J. S. McKenzie, & J. Féger (eds.), *The basal ganglia IV* (Vol. 41, pp. 95–107). Boston, MA: Springer US. https://doi.org/10.1007/978-1-4613-0485-2_9

Averbeck, B. B., & Costa, V. D. (2017). Motivational neural circuits underlying reinforcement learning. *Nature Neuroscience*, *20*(4), 505–512. https://doi.org/10.1038/nn.4506

Bas-Hoogendam, J. M., van Steenbergen, H., van der Wee, N. J. A., & Westenberg, P. M. (2020). Amygdala hyperreactivity to faces conditioned with a social-evaluative meaning-a multiplex, multigenerational fMRI study on social anxiety endophenotypes. *NeuroImage. Clinical*, *26*, 102247. https://doi.org/10.1016/j.nicl.2020.102247

Baumgartner, H. M., Cole, S. L., Olney, J. J., & Berridge, K. C. (2020). Desire or Dread from Nucleus Accumbens Inhibitions: Reversed by Same-Site Optogenetic Excitations. *The Journal of Neuroscience*, *40*(13), 2737–2752. https://doi.org/10.1523/JNEUROSCI.2902-19.2020

Baumgartner, H. M., Schulkin, J., & Berridge, K. C. (2021). Activating Corticotropin-Releasing Factor Systems in the Nucleus Accumbens, Amygdala, and Bed Nucleus of Stria Terminalis: Incentive Motivation or Aversive Motivation? *Biological Psychiatry*, *89*(12), 1162–1175. https://doi.org/10.1016/j.biopsych.2021.01.007

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews. Neuroscience*, *3*(7), 563–573. https://doi.org/10.1038/nrn875

Bechara, A., Berridge, K. C., Bickel, W. K., Morón, J. A., Williams, S. B., & Stein, J. S. (2019). A neurobehavioral approach to addiction: implications for the opioid epidemic and the psychology of addiction. *Psychological Science in the Public Interest*, *20*(2), 96–127. https://doi.org/10.1177/1529100619860513

Becker, J. B. (2016). Sex differences in addiction. *Dialogues in Clinical Neuroscience*, *18*(4), 395–402.

Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, *29*(1), 36–47. https://doi.org/10.1016/j.yfrne.2007.07.003

Bergan, J. F., Ben-Shaul, Y., & Dulac, C. (2014). Sex-specific processing of social cues in the medial amygdala. *eLife*, *3*, e02743. https://doi.org/10.7554/eLife.02743

Berridge, K C, & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. https://doi.org/10.1016/S0166-2236(03)00233-9

Berridge, K., & Winkielman, P. (2003). What is an unconscious emotion?(The case for unconscious "liking"). *Cognition & emotion*, *17*(2), 181–211. https://doi.org/10.1080/02699930302289

Berridge, Kent C. (2012). From prediction error to incentive salience: mesolimbic computation of reward motivation. *The European Journal of Neuroscience*, *35*(7), 1124–1143. https://doi.org/10.1111/j.1460-9568.2012.07990.x

Berridge, Kent C. (2019). Affective valence in the brain: modules or modes? *Nature Reviews. Neuroscience*, *20*(4), 225–234. https://doi.org/10.1038/s41583-019-0122-8

Berridge, Kent C, & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *The American Psychologist*, *71*(8), 670–679. https://doi.org/10.1037/amp0000059

Beyeler, A., Chang, C.-J., Silvestre, M., Lévêque, C., Namburi, P., Wildes, C. P., & Tye, K. M. (2018). Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. *Cell reports*, *22*(4), 905–918. https://doi.org/10.1016/j.celrep.2017.12.097

Bindra, D. (1978). How adaptive behavior is produced: a perceptual-motivational alternative to response reinforcements. *Behavioral and Brain Sciences*, *1*(01), 41. https://doi.org/10.1017/S0140525X00059380

Cai, H., Haubensak, W., Anthony, T. E., & Anderson, D. J. (2014). Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature Neuroscience*, *17*(9), 1240–1248. https://doi.org/10.1038/nn.3767

Canteras, N. S., Simerly, R. B., & Swanson, L. W. (1995). Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *The Journal of Comparative Neurology*, *360*(2), 213–245. https://doi.org/10.1002/cne.903600203

Castro, D. C., & Berridge, K. C. (2017). Opioid and orexin hedonic hotspots in rat orbitofrontal cortex and insula. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(43), E9125–E9134. https://doi.org/10.1073/pnas.1705753114

Chen, P. B., Hu, R. K., Wu, Y. E., Pan, L., Huang, S., Micevych, P. E., & Hong, W. (2019). Sexually dimorphic control of parenting behavior by the medial amygdala. *Cell*, *176*(5), 1206–1221.e18. https://doi.org/10.1016/j.cell.2019.01.024

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., … Lüthi, A. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature*, *468*(7321), 277–282. https://doi.org/10.1038/nature09559

Claus, E. D., Ewing, S. W. F., Filbey, F. M., Sabbineni, A., & Hutchison, K. E. (2011). Identifying neurobiological phenotypes associated with alcohol use disorder severity. *Neuropsychopharmacology*, *36*(10), 2086–2096. https://doi.org/10.1038/npp.2011.99

Cooke, B. M., & Woolley, C. S. (2005). Sexually dimorphic synaptic organization of the medial amygdala. *The Journal of Neuroscience*, *25*(46), 10759–10767. https://doi.org/10.1523/JNEUROSCI.2919-05.2005

Corbit, L. H., & Janak, P. H. (2007). Ethanol-associated cues produce general pavlovian-instrumental transfer. *Alcoholism, Clinical and Experimental Research*, *31*(5), 766–774. https://doi.org/10.1111/j.1530-0277.2007.00359.x

Dabrowska, J., Hazra, R., Guo, J.-D., Dewitt, S., & Rainnie, D. G. (2013). Central CRF neurons are not created equal: phenotypic differences in CRF-containing neurons of the rat paraventricular hypothalamus and the bed nucleus of the stria terminalis. *Frontiers in Neuroscience*, *7*, 156. https://doi.org/10.3389/fnins.2013.00156

De Boer, S. F., & Koolhaas, J. M. (2003). Defensive burying in rodents: ethology, neurobiology and psychopharmacology. *European Journal of Pharmacology*, *463*(1-3), 145–161. https://doi.org/10.1016/s0014-2999(03)01278-0

De Olmos, J. S., & Ingram, W. R. (1972). The projection field of the stria terminalis in the rat brain. An experimental study. *The Journal of Comparative Neurology*, *146*(3), 303–334. https://doi.org/10.1002/cne.901460303

de Olmos, J. S., & Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. *Annals of the New York Academy of Sciences*, *877*, 1–32. https://doi.org/10.1111/j.1749-6632.1999.tb09258.x

Deng, H., Xiao, X., & Wang, Z. (2016). Periaqueductal gray neuronal activities underlie different aspects of defensive behaviors. *The Journal of Neuroscience*, *36*(29), 7580–7588. https://doi.org/10.1523/JNEUROSCI.4425-15.2016

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science*, *305*(5686), 1014–1017. https://doi.org/10.1126/science.1099020

DiBenedictis, B. T., Helfand, A. I., Baum, M. J., & Cherry, J. A. (2014). A quantitative comparison of the efferent projections of the anterior and posterior subdivisions of the medial amygdala in female mice. *Brain Research*, *1543*, 101–108. https://doi.org/10.1016/j.brainres.2013.10.046

DiFeliceantonio, A. G., & Berridge, K. C. (2012). Which cue to "want"? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. *Behavioural Brain Research*, *230*(2), 399–408. https://doi.org/10.1016/j.bbr.2012.02.032

Donegan, N. H., Sanislow, C. A., Blumberg, H. P., Fulbright, R. K., Lacadie, C., Skudlarski, P., … Wexler, B. E. (2003). Amygdala hyperreactivity in borderline personality disorder: implications for emotional dysregulation. *Biological Psychiatry*, *54*(11), 1284–1293. https://doi.org/10.1016/s0006-3223(03)00636-x

Dong, H. W., Petrovich, G. D., & Swanson, L. W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Research. Brain Research Reviews*, *38*(1-2), 192–246. https://doi.org/10.1016/S0165-0173(01)00079-0

Edmondson, A. J., Brennan, C. A., & House, A. O. (2016). Non-suicidal reasons for self-harm: A systematic review of self-reported accounts. *Journal of Affective Disorders*, *191*, 109–117. https://doi.org/10.1016/j.jad.2015.11.043

Fadok, J. P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., … Lüthi, A. (2017). A competitive inhibitory circuit for selection of active and passive fear responses. *Nature*, *542*(7639), 96–100. https://doi.org/10.1038/nature21047

Fadok, J. P., Markovic, M., Tovote, P., & Lüthi, A. (2018). New perspectives on central amygdala function. *Current Opinion in Neurobiology*, *49*, 141–147. https://doi.org/10.1016/j.conb.2018.02.009

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *The Pavlovian journal of biological science*, *15*(4), 177–182. https://doi.org/10.1007/BF03001163

Faure, A., Reynolds, S. M., Richard, J. M., & Berridge, K. C. (2008). Mesolimbic dopamine in desire and dread: enabling motivation to be generated by localized glutamate disruptions in nucleus accumbens. *The Journal of Neuroscience*, *28*(28), 7184–7192. https://doi.org/10.1523/JNEUROSCI.4961-07.2008

Faure, A., Richard, J. M., & Berridge, K. C. (2010). Desire and dread from the nucleus accumbens: cortical glutamate and subcortical GABA differentially generate motivation and hedonic impact in the rat. *Plos One*, *5*(6), e11223. https://doi.org/10.1371/journal.pone.0011223

Fergani, C., McCarthy, E., Leon, S., & Navarro, V. (2019). SAT-405 Activation of Kiss1 Neurons in the Posterodorsal Medial Amygdala Stimulates Gonadotropin Release via the Release of Kisspeptin in Female Mice. *Journal of the Endocrine Society*, *3*(Supplement_1). https://doi.org/10.1210/js.2019-SAT-405

Ferguson, J. N., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience*, *21*(20), 8278–8285. https://doi.org/10.1523/JNEUROSCI.21-20-08278.2001

Flagel, S. B., Akil, H., & Robinson, T. E. (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. *Neuropharmacology*, *56 Suppl 1*, 139–148. https://doi.org/10.1016/j.neuropharm.2008.06.027

Frankiensztajn, L. M., Gur-Pollack, R., & Wagner, S. (2018). A combinatorial modulation of synaptic plasticity in the rat medial amygdala by oxytocin, urocortin3 and estrogen. *Psychoneuroendocrinology*, *92*, 95–102. https://doi.org/10.1016/j.psyneuen.2018.04.006

Funk, C. K., O'Dell, L. E., Crawford, E. F., & Koob, G. F. (2006). Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. *The Journal of Neuroscience*, *26*(44), 11324–11332. https://doi.org/10.1523/JNEUROSCI.3096-06.2006

Funk, D., Li, Z., & Lê, A. D. (2006). Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. *Neuroscience*, *138*(1), 235–243. https://doi.org/10.1016/j.neuroscience.2005.10.062

Garbusow, M., Nebe, S., Sommer, C., Kuitunen-Paul, S., Sebold, M., Schad, D. J., … Heinz, A. (2019). Pavlovian-To-Instrumental Transfer and Alcohol Consumption in Young Male Social Drinkers: Behavioral, Neural and Polygenic Correlates. *Journal of clinical medicine*, *8*(8). https://doi.org/10.3390/jcm8081188

Gomez, D. M., & Newman, S. W. (1992). Differential projections of the anterior and posterior regions of the medial amygdaloid nucleus in the Syrian hamster. *The Journal of Comparative Neurology*, *317*(2), 195–218. https://doi.org/10.1002/cne.903170208

Goode, T. D., Ressler, R. L., Acca, G. M., Miles, O. W., & Maren, S. (2019). Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. *eLife*, *8*. https://doi.org/10.7554/eLife.46525

Hagihara, K. M., Bukalo, O., Zeller, M., Aksoy-Aksel, A., Karalis, N., Limoges, A., … Holmes, A. (2021). Intercalated amygdala clusters orchestrate a switch in fear state. *Nature*. https://doi.org/10.1038/s41586-021-03593-1

Haight, J. L., Fuller, Z. L., Fraser, K. M., & Flagel, S. B. (2017). A food-predictive cue attributed with incentive salience engages subcortical afferents and efferents of the paraventricular nucleus of the thalamus. *Neuroscience*, *340*, 135–152. https://doi.org/10.1016/j.neuroscience.2016.10.043

Han, W., Tellez, L. A., Rangel, M. J., Motta, S. C., Zhang, X., Perez, I. O., … de Araujo, I. E. (2017). Integrated control of predatory hunting by the central nucleus of the amygdala. *Cell*, *168*(1-2), 311–324.e18. https://doi.org/10.1016/j.cell.2016.12.027

Hariri, A. R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., & Weinberger, D. R. (2005). A susceptibility gene for affective disorders and the response of the human amygdala. *Archives of General Psychiatry*, *62*(2), 146–152. https://doi.org/10.1001/archpsyc.62.2.146

Haubensak, W., Kunwar, P. S., Cai, H., Ciocchi, S., Wall, N. R., Ponnusamy, R., … Anderson, D. J. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature*, *468*(7321), 270–276. https://doi.org/10.1038/nature09553

Heimer, L, Harlan, R. E., Alheid, G. F., Garcia, M. M., & de Olmos, J. (1997). Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. *Neuroscience*, *76*(4), 957–1006. https://doi.org/10.1016/s0306-4522(96)00405-8

Heimer, Lennart, & Van Hoesen, G. W. (2006). The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neuroscience and Biobehavioral Reviews*, *30*(2), 126–147. https://doi.org/10.1016/j.neubiorev.2005.06.006

Heimer, Lennart, Van Hoesen, G. W., Trimble, M., & Zahm, D. S. (2007). *Anatomy of Neuropsychiatry: The New Anatomy of the Basal Forebrain and Its Implications for Neuropsychiatric Illness*. Academic Press.

Herrick, C. J. (1926). Neurological' ' foundations of animal behavior. In *Brains' ' of rats and men.* (p. 382). Chicago: University of Chicago Press.

Heyman, G. M., & Dunn, B. (2002). Decision biases and persistent illicit drug use: an experimental study of distributed choice and addiction. *Drug and Alcohol Dependence*, *67*(2), 193–203.

Hiura, L. C., Kelly, A. M., & Ophir, A. G. (2018). Age-specific and context-specific responses of the medial extended amygdala in the developing prairie vole. *Developmental Neurobiology*, *78*(12), 1231–1245. https://doi.org/10.1002/dneu.22648

Holland, P. C., & Gallagher, M. (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *The European Journal of Neuroscience*, *17*(8), 1680–1694. https://doi.org/10.1046/j.1460-9568.2003.02585.x

Holmes, N. M., Marchand, A. R., & Coutureau, E. (2010). Pavlovian to instrumental transfer: a neurobehavioural perspective. *Neuroscience and Biobehavioral Reviews*, *34*(8), 1277–1295. https://doi.org/10.1016/j.neubiorev.2010.03.007

Hong, W., Kim, D.-W., & Anderson, D. J. (2014). Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. *Cell*, *158*(6), 1348–1361. https://doi.org/10.1016/j.cell.2014.07.049

Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III--the final common pathway. *Schizophrenia Bulletin*, *35*(3), 549–562. https://doi.org/10.1093/schbul/sbp006

Hu, M., & Becker, J. B. (2008). Acquisition of cocaine self-administration in ovariectomized female rats: effect of estradiol dose or chronic estradiol administration. *Drug and Alcohol Dependence*, *94*(1-3), 56–62. https://doi.org/10.1016/j.drugalcdep.2007.10.005

Hu, M., Crombag, H. S., Robinson, T. E., & Becker, J. B. (2004). Biological basis of sex differences in the propensity to self-administer cocaine. *Neuropsychopharmacology*, *29*(1), 81–85. https://doi.org/10.1038/sj.npp.1300301

Hu, R. K., Zuo, Y., Ly, T., Wang, J., Meera, P., Wu, Y. E., & Hong, W. (2021). An amygdala-to-hypothalamus circuit for social reward. *Nature Neuroscience*. https://doi.org/10.1038/s41593-021-00828-2

Jackson, L. R., Robinson, T. E., & Becker, J. B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology*, *31*(1), 129–138. https://doi.org/10.1038/sj.npp.1300778

James, M. K., Feldman, P. L., Schuster, S. V., Bilotta, J. M., Brackeen, M. F., & Leighton, H. J. (1991). Opioid receptor activity of GI 87084B, a novel ultra-short acting analgesic, in isolated tissues. *The Journal of Pharmacology and Experimental Therapeutics*, *259*(2), 712–718.

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. https://doi.org/10.1038/nature12041

Jiang, Z., Rajamanickam, S., & Justice, N. J. (2019). CRF signaling between neurons in the paraventricular nucleus of the hypothalamus (PVN) coordinates stress responses. *Neurobiology of stress*, *11*, 100192. https://doi.org/10.1016/j.ynstr.2019.100192

Johnston, J. B. (1923). Further contributions to the study of the evolution of the forebrain. In H. H. Donaldson, J. B. Johnston, A. Meyer, O. S. Strong, & C. J. Herrick (eds.), *Journal of Comparative Neurology, Volume 35* (Vol. 35, pp. 337–482). Wistar Institute of Anatomy and Biology.

Joos, A. A. B., Saum, B., van Elst, L. T., Perlov, E., Glauche, V., Hartmann, A., … Zeeck, A. (2011). Amygdala hyperreactivity in restrictive anorexia nervosa. *Psychiatry Research*, *191*(3), 189–195. https://doi.org/10.1016/j.pscychresns.2010.11.008

Kelley, A. E., & Berridge, K. C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. *The Journal of Neuroscience*, *22*(9), 3306–3311. https://doi.org/20026361

Kikusui, T., Kajita, M., Otsuka, N., Hattori, T., Kumazawa, K., Watarai, A., … Mogi, K. (2018). Sex differences in olfactory-induced neural activation of the amygdala. *Behavioural Brain Research*, *346*, 96–104. https://doi.org/10.1016/j.bbr.2017.11.034

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in Pavlovian fear conditioning: a critical review. *Neuroscience and Biobehavioral Reviews*, *30*(2), 188–202. https://doi.org/10.1016/j.neubiorev.2005.06.005

Kim, J., Zhang, X., Muralidhar, S., LeBlanc, S. A., & Tonegawa, S. (2017). Basolateral to central amygdala neural circuits for appetitive behaviors. *Neuron*, *93*(6), 1464–1479.e5. https://doi.org/10.1016/j.neuron.2017.02.034

Kochli, D. E., Thompson, E. C., Fricke, E. A., Postle, A. F., & Quinn, J. J. (2015). The amygdala is critical for trace, delay, and contextual fear conditioning. *Learning & Memory*, *22*(2), 92–100. https://doi.org/10.1101/lm.034918.114

Kollack-Walker, S., & Newman, S. W. (1997). Mating-induced expression of c-fos in the male Syrian hamster brain: Role of experience, pheromones, and ejaculations. *Journal of Neurobiology*, *32*(5), 481–501. https://doi.org/10.1002/(SICI)1097-4695(199705)32:5<481::AID-NEU4>3.0.CO;2-1

Kollack-Walker, S., & Newman, S. W. (n.d.). Mating-Induced Expression of c-fos in the Male Syrian Hamster Brain: Role of Experience, Pheromones, and Ejaculations. Retrieved July 29, 2021, from https://onlinelibrary.wiley.com/doi/pdf/10.1002/%28SICI%291097-4695%28199705%2932%3A5%3C481%3A%3AAID-NEU4%3E3.0.CO%3B2-1?casa_token=AWasRCBofzwAAAAA:ISeK5Ge-dOAWFODQPoF5TbT4aVNUADJC-v28GGSxiQQ_qbVl0m0d713Mp6K8AtD_3wynxlvqWgUn7w

Koob, G. F. (2010). The role of CRF and CRF-related peptides in the dark side of addiction. *Brain Research*, *1314*, 3–14. https://doi.org/10.1016/j.brainres.2009.11.008

Koob, G. F. (2013). Addiction is a Reward Deficit and Stress Surfeit Disorder. *Frontiers in psychiatry*, *4*, 72. https://doi.org/10.3389/fpsyt.2013.00072

Koob, G. F., Buck, C. L., Cohen, A., Edwards, S., Park, P. E., Schlosburg, J. E., … George, O. (2014). Addiction as a stress surfeit disorder. *Neuropharmacology*, *76 Pt B*, 370–382. https://doi.org/10.1016/j.neuropharm.2013.05.024

Koob, G. F., & Le Moal, M. (2008). Addiction and the brain antireward system. *Annual Review of Psychology*, *59*, 29–53. https://doi.org/10.1146/annurev.psych.59.103006.093548

Koob, G. F., & Schulkin, J. (2019). Addiction and stress: An allostatic view. *Neuroscience and Biobehavioral Reviews*, *106*, 245–262. https://doi.org/10.1016/j.neubiorev.2018.09.008

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–238. https://doi.org/10.1038/npp.2009.110

Kravitz, A. V., & Kreitzer, A. C. (2011). Optogenetic manipulation of neural circuitry in vivo. *Current Opinion in Neurobiology*, *21*(3), 433–439. https://doi.org/10.1016/j.conb.2011.02.010

Kringelbach, M. L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nature Reviews. Neuroscience*, *6*(9), 691–702. https://doi.org/10.1038/nrn1747

LeBlanc, K. H., Ostlund, S. B., & Maidment, N. T. (2012). Pavlovian-to-instrumental transfer in cocaine seeking rats. *Behavioral Neuroscience*, *126*(5), 681–689. https://doi.org/10.1037/a0029534

LeDoux, J. (2007). The amygdala. *Current Biology*, *17*(20), R868–74. https://doi.org/10.1016/j.cub.2007.08.005

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *The Journal of Neuroscience*, *8*(7), 2517–2529. https://doi.org/10.1523/JNEUROSCI.08-07-02517.1988

Lenoir, M., Augier, E., Vouillac, C., & Ahmed, S. H. (2013). A choice-based screening method for compulsive drug users in rats. *Current Protocols in Neuroscience*, *Chapter 9*, Unit 9.44. https://doi.org/10.1002/0471142301.ns0944s64

Lenoir, M., Serre, F., Cantin, L., & Ahmed, S. H. (2007). Intense sweetness surpasses cocaine reward. *Plos One*, *2*(8), e698. https://doi.org/10.1371/journal.pone.0000698

Lesscher, H. M. B., & Vanderschuren, L. J. M. J. (2012). Compulsive drug use and its neural substrates. *Reviews in the neurosciences*, *23*(5-6), 731–745. https://doi.org/10.1515/revneuro-2012-0066

Li, C., & Krashes, M. J. (2016). Foraging and flight trump defense and fight. *Nature Neuroscience*, *19*(5), 645–646. https://doi.org/10.1038/nn.4294

Li, Y., Mathis, A., Grewe, B. F., Osterhout, J. A., Ahanonu, B., Schnitzer, M. J., … Dulac, C. (2017). Neuronal representation of social information in the medial amygdala of awake behaving mice. *Cell*, *171*(5), 1176–1190.e17. https://doi.org/10.1016/j.cell.2017.10.015

Mahler, S. V., & Berridge, K. C. (2009). Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. *The Journal of Neuroscience*, *29*(20), 6500–6513. https://doi.org/10.1523/JNEUROSCI.3875-08.2009

Mahler, S. V., & Berridge, K. C. (2012). What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. *Psychopharmacology*, *221*(3), 407–426. https://doi.org/10.1007/s00213-011-2588-6

Marcinkiewcz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., … Kash, T. L. (2016). Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature*, *537*(7618), 97–101. https://doi.org/10.1038/nature19318

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience*, *24*, 897–931. https://doi.org/10.1146/annurev.neuro.24.1.897

Matos, H. Y., Hernandez-Pineda, D., Charpentier, C. M., Rusk, A., Corbin, J. G., & Jones, K. S. (2020). Sex Differences in Biophysical Signatures across Molecularly Defined Medial Amygdala Neuronal Subpopulations. *eNeuro*, *7*(4). https://doi.org/10.1523/ENEURO.0035-20.2020

Merchenthaler, I. (1984). Corticotropin releasing factor (CRF)-like immunoreactivity in the rat central nervous system. Extrahypothalamic distribution. *Peptides*, *5*, 53–69. https://doi.org/10.1016/0196-9781(84)90265-1

Michelsen, L. G., & Hug, C. C. (1996). The pharmacokinetics of remifentanil. *Journal of Clinical Anesthesia*, *8*(8), 679–682. https://doi.org/10.1016/s0952-8180(96)00179-1

Miller, S. M., Marcotulli, D., Shen, A., & Zweifel, L. S. (2019). Divergent medial amygdala projections regulate approach-avoidance conflict behavior. *Nature Neuroscience*, *22*(4), 565–575. https://doi.org/10.1038/s41593-019-0337-z

Moran-Santa Maria, M. M., Flanagan, J., & Brady, K. (2014). Ovarian hormones and drug abuse. *Current Psychiatry Reports*, *16*(11), 511. https://doi.org/10.1007/s11920-014-0511-7

Nair, S. G., Gray, S. M., & Ghitza, U. E. (2006). Role of food type in yohimbine- and pellet-priming-induced reinstatement of food seeking. *Physiology & Behavior*, *88*(4-5), 559–566. https://doi.org/10.1016/j.physbeh.2006.05.014

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Annals of the New York Academy of Sciences*, *877*, 242–257. https://doi.org/10.1111/j.1749-6632.1999.tb09271.x

Nordman, J. C., Ma, X., Gu, Q., Potegal, M., Li, H., Kravitz, A. V., & Li, Z. (2020). Potentiation of Divergent Medial Amygdala Pathways Drives Experience-Dependent Aggression Escalation. *The Journal of Neuroscience*, *40*(25), 4858–4880. https://doi.org/10.1523/JNEUROSCI.0370-20.2020

Nordman, J., & Li, Z. (2020). The Dorsal Raphe Regulates the Duration of Attack through the Medial Orbitofrontal Cortex and Medial Amygdala. *eNeuro*, *7*(5). https://doi.org/10.1523/ENEURO.0331-20.2020

Nordman, J., Ma, X., & Li, Z. (2020). Traumatic Stress Induces Prolonged Aggression Increase through Synaptic Potentiation in the Medial Amygdala Circuits. *eNeuro*, *7*(4). https://doi.org/10.1523/ENEURO.0147-20.2020

Olney, J. J., Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2018). Current perspectives on incentive salience and applications to clinical disorders. *Current Opinion in Behavioral Sciences*, *22*, 59–69. https://doi.org/10.1016/j.cobeha.2018.01.007

Olsson, M., Björklund, A., & Campbell, K. (1998). Early specification of striatal projection neurons and interneuronal subtypes in the lateral and medial ganglionic eminence. *Neuroscience*, *84*(3), 867–876. https://doi.org/10.1016/s0306-4522(97)00532-0

Otto, T., Cousens, G., & Rajewski, K. (1997). Odor-guided fear conditioning in rats: 1. Acquisition, retention, and latent inhibition. *Behavioral Neuroscience*, *111*(6), 1257–1264.

Padilla, S. L., Qiu, J., Nestor, C. C., Zhang, C., Smith, A. W., Whiddon, B. B., … Palmiter, R. D. (2017). AgRP to Kiss1 neuron signaling links nutritional state and fertility. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(9), 2413–2418. https://doi.org/10.1073/pnas.1621065114

Padilla, S. L., Qiu, J., Soden, M. E., Sanz, E., Nestor, C. C., Barker, F. D., … Palmiter, R. D. (2016). Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. *Nature Neuroscience*, *19*(5), 734–741. https://doi.org/10.1038/nn.4274

Paretkar, T., & Dimitrov, E. (2019). Activation of enkephalinergic (Enk) interneurons in the central amygdala (CeA) buffers the behavioral effects of persistent pain. *Neurobiology of Disease*, *124*, 364–372. https://doi.org/10.1016/j.nbd.2018.12.005

Paxinos, G., & Watson, C. (2007). The rat brain in stereotaxic coordinates, Ed 6. Amsterdam: Academic.

Peciña, S, & Berridge, K. C. (2000). Opioid site in nucleus accumbens shell mediates eating and hedonic “liking” for food: map based on microinjection Fos plumes. *Brain Research*, *863*(1-2), 71–86. https://doi.org/10.1016/s0006-8993(00)02102-8

Peciña, Susana, & Berridge, K. C. (2013). Dopamine or opioid stimulation of nucleus accumbens similarly amplify cue-triggered “wanting” for reward: entire core and medial shell mapped as substrates for PIT enhancement. *The European Journal of Neuroscience*, *37*(9), 1529–1540. https://doi.org/10.1111/ejn.12174

Peciña, Susana, Schulkin, J., & Berridge, K. C. (2006). Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical

positive incentive effects in stress? *BMC Biology*, *4*, 8. https://doi.org/10.1186/1741-7007-4-8

Penzo, M. A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., … Li, B. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature*, *519*(7544), 455–459. https://doi.org/10.1038/nature13978

Petrovich, G. D., Canteras, N. S., & Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research. Brain Research Reviews*, *38*(1-2), 247–289. https://doi.org/10.1016/S0165-0173(01)00080-7

Pomrenze, M. B., Millan, E. Z., Hopf, F. W., Keiflin, R., Maiya, R., Blasio, A., … Messing, R. O. (2015). A transgenic rat for investigating the anatomy and function of corticotrophin releasing factor circuits. *Frontiers in Neuroscience*, *9*, 487. https://doi.org/10.3389/fnins.2015.00487

Raam, T., & Hong, W. (2021). Organization of neural circuits underlying social behavior: A consideration of the medial amygdala. *Current Opinion in Neurobiology*, *68*, 124–136. https://doi.org/10.1016/j.conb.2021.02.008

Rasia-Filho, A. A., Fabian, C., Rigoti, K. M., & Achaval, M. (2004). Influence of sex, estrous cycle and motherhood on dendritic spine density in the rat medial amygdala revealed by the Golgi method. *Neuroscience*, *126*(4), 839–847. https://doi.org/10.1016/j.neuroscience.2004.04.009

Rasmussen, S., Hawton, K., Philpott-Morgan, S., & O'Connor, R. C. (2016). Why Do Adolescents Self-Harm? *Crisis*, *37*(3), 176–183. https://doi.org/10.1027/0227-5910/a000369

Reynolds, S. M., & Berridge, K. C. (2001). Fear and feeding in the nucleus accumbens shell: rostrocaudal segregation of GABA-elicited defensive behavior versus eating behavior. *The Journal of Neuroscience*, *21*(9), 3261–3270.

Richard, J. M., & Berridge, K. C. (2011a). Metabotropic glutamate receptor blockade in nucleus accumbens shell shifts affective valence towards fear and disgust. *The European Journal of Neuroscience*, *33*(4), 736–747. https://doi.org/10.1111/j.1460-9568.2010.07553.x

Richard, J. M., & Berridge, K. C. (2011b). Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but D(1) and D(2) together for fear. *The Journal of Neuroscience*, *31*(36), 12866–12879. https://doi.org/10.1523/JNEUROSCI.1339-11.2011

Richard, J. M., Plawecki, A. M., & Berridge, K. C. (2013). Nucleus accumbens GABAergic inhibition generates intense eating and fear that resists environmental retuning and needs no local dopamine. *The European Journal of Neuroscience*, *37*(11), 1789–1802. https://doi.org/10.1111/ejn.12194

Richardson, N. R., & Roberts, D. C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*, *66*(1), 1–11. https://doi.org/10.1016/0165-0270(95)00153-0

Robbins, T. W., Watson, B. A., Gaskin, M., & Ennis, C. (1983). Contrasting interactions of pipradrol, d-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. *Psychopharmacology*, *80*(2), 113–119.

Robinson, M. J. F., & Berridge, K. C. (2013). Instant transformation of learned repulsion into motivational "wanting". *Current Biology*, *23*(4), 282–289. https://doi.org/10.1016/j.cub.2013.01.016

Robinson, M. J. F., Warlow, S. M., & Berridge, K. C. (2014). Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. *The Journal of Neuroscience*, *34*(50), 16567–16580. https://doi.org/10.1523/JNEUROSCI.2013-14.2014

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research. Brain Research Reviews*, *18*(3), 247–291. https://doi.org/10.1016/0165-0173(93)90013-P

Sandy, P. T. (2013). Motives for self-harm: views of nurses in a secure unit. *International nursing review*, *60*(3), 358–365. https://doi.org/10.1111/inr.12038

Servonnet, A., Hernandez, G., El Hage, C., Rompré, P.-P., & Samaha, A.-N. (2020). Optogenetic activation of the basolateral amygdala promotes both appetitive conditioning and the instrumental pursuit of reward cues. *The Journal of Neuroscience*, *40*(8), 1732–1743. https://doi.org/10.1523/JNEUROSCI.2196-19.2020

Smith, D. M., & Torregrossa, M. M. (2021). Valence encoding in the amygdala influences motivated behavior. *Behavioural Brain Research*, 113370. https://doi.org/10.1016/j.bbr.2021.113370

Specio, S. E., Wee, S., O'Dell, L. E., Boutrel, B., Zorrilla, E. P., & Koob, G. F. (2008). CRF(1) receptor antagonists attenuate escalated cocaine self-administration in rats. *Psychopharmacology*, *196*(3), 473–482. https://doi.org/10.1007/s00213-007-0983-9

Steinberg, E. E., Gore, F., Heifets, B. D., Taylor, M. D., Norville, Z. C., Beier, K. T., … Malenka, R. C. (2020). Amygdala-Midbrain Connections Modulate Appetitive and Aversive Learning. *Neuron*, *106*(6), 1026–1043.e9. https://doi.org/10.1016/j.neuron.2020.03.016

Stolzenberg, D. S., & Mayer, H. S. (2019). Experience-dependent mechanisms in the regulation of parental care. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2019.04.002

Swalve, N., Smethells, J. R., & Carroll, M. E. (2016). Sex differences in the acquisition and maintenance of cocaine and nicotine self-administration in rats. *Psychopharmacology*, *233*(6), 1005–1013. https://doi.org/10.1007/s00213-015-4183-8

Swanson, L W. (2000). Cerebral hemisphere regulation of motivated behavior. *Brain Research*, *886*(1-2), 113–164. https://doi.org/10.1016/s0006-8993(00)02905-x

Swanson, L W, & Petrovich, G. D. (1998). What is the amygdala? *Trends in Neurosciences*, *21*(8), 323–331. https://doi.org/10.1016/s0166-2236(98)01265-x

Swanson, L W, Sawchenko, P. E., Rivier, J., & Vale, W. W. (1983). Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology*, *36*(3), 165–186. https://doi.org/10.1159/000123454

Swanson, Larry W. (2005). Anatomy of the soul as reflected in the cerebral hemispheres: neural circuits underlying voluntary control of basic motivated behaviors. *The Journal of Comparative Neurology*, *493*(1), 122–131. https://doi.org/10.1002/cne.20733

Takahashi, L. K., Hubbard, D. T., Lee, I., Dar, Y., & Sipes, S. M. (2007). Predator odor-induced conditioned fear involves the basolateral and medial amygdala. *Behavioral Neuroscience*, *121*(1), 100–110. https://doi.org/10.1037/0735-7044.121.1.100

Terner, J. M., & de Wit, H. (2006). Menstrual cycle phase and responses to drugs of abuse in humans. *Drug and Alcohol Dependence*, *84*(1), 1–13. https://doi.org/10.1016/j.drugalcdep.2005.12.007

Thorpe, D., Lacy, R. T., & Strickland, J. C. (2020). Acquisition of remifentanil self-administration: Enhanced in female rats but no effect of adolescent stress exposure. *Pharmacology, Biochemistry, and Behavior*, *199*, 173038. https://doi.org/10.1016/j.pbb.2020.173038

Tom, R. L., Ahuja, A., Maniates, H., Freeland, C. M., & Robinson, M. J. F. (2019). Optogenetic activation of the central amygdala generates addiction-like preference for reward. *The European Journal of Neuroscience*, *50*(3), 2086–2100. https://doi.org/10.1111/ejn.13967

Tomie, A., Grimes, K. L., & Pohorecky, L. A. (2008). Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. *Brain Research Reviews*, *58*(1), 121–135. https://doi.org/10.1016/j.brainresrev.2007.12.003

Torruella-Suárez, M. L., Vandenberg, J. R., Cogan, E. S., Tipton, G. J., Teklezghi, A., Dange, K., … McElligott, Z. A. (2020). Manipulations of Central Amygdala Neurotensin Neurons Alter the Consumption of Ethanol and Sweet Fluids in Mice. *The Journal of Neuroscience*, *40*(3), 632–647. https://doi.org/10.1523/JNEUROSCI.1466-19.2019

Treit, D., Pinel, J. P., & Fibiger, H. C. (1981). Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharmacology, Biochemistry, and Behavior*, *15*(4), 619–626. https://doi.org/10.1016/0091-3057(81)90219-7

Tye, K. M. (2018). Neural circuit motifs in valence processing. *Neuron*, *100*(2), 436–452. https://doi.org/10.1016/j.neuron.2018.10.001

Vanderschuren, L. J. M. J., & Everitt, B. J. (2004). Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science*, *305*(5686), 1017–1019. https://doi.org/10.1126/science.1098975

Venniro, M., Caprioli, D., Zhang, M., Whitaker, L. R., Zhang, S., Warren, B. L., … Shaham, Y. (2017). The Anterior Insular Cortex→Central Amygdala Glutamatergic Pathway Is Critical to Relapse after Contingency Management. *Neuron*, *96*(2), 414–427.e8. https://doi.org/10.1016/j.neuron.2017.09.024

Venniro, M., Russell, T. I., Ramsey, L. A., Richie, C. T., Lesscher, H. M. B., Giovanetti, S. M., … Shaham, Y. (2020). Abstinence-dependent dissociable central amygdala microcircuits control drug craving. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(14), 8126–8134. https://doi.org/10.1073/pnas.2001615117

Warlow, S. M., Baumgartner, H. M., Freeland, C. M., Naffziger, E. E., Olney, J. J., Berridge, K. C., & Robinson, M. J. (2020). *The Cambridge Handbook of Substance and Behavioral Addictions*. (S. Sussman, ed.). Cambridge University Press.

Warlow, S. M., & Berridge, K. C. (2021). Incentive motivation: "wanting" roles of central amygdala circuitry. *Behavioural Brain Research*, *411*, 113376. https://doi.org/10.1016/j.bbr.2021.113376

Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2020). The central amygdala recruits mesocorticolimbic circuitry for pursuit of reward or pain. *Nature Communications*, *11*(1), 2716. https://doi.org/10.1038/s41467-020-16407-1

Warlow, S. M., Robinson, M. J. F., & Berridge, K. C. (2017). Optogenetic central amygdala stimulation intensifies and narrows motivation for cocaine. *The Journal of Neuroscience*, *37*(35), 8330–8348. https://doi.org/10.1523/JNEUROSCI.3141-16.2017

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *Journal of comparative and physiological psychology*, *49*(4), 381–391. https://doi.org/10.1037/h0088009

Westberry, J. M., & Meredith, M. (2017). Characteristic response to chemosensory signals in gabaergic cells of medial amygdala is not driven by main olfactory input. *Chemical Senses*, *42*(1), 13–24. https://doi.org/10.1093/chemse/bjw096

Wilson, T. D., Valdivia, S., Khan, A., Ahn, H.-S., Adke, A. P., Martinez Gonzalez, S., … Carrasquillo, Y. (2019). Dual and opposing functions of the central amygdala in the modulation of pain. *Cell reports*, *29*(2), 332–346.e5. https://doi.org/10.1016/j.celrep.2019.09.011

Wolff, S. B. E., Gründemann, J., Tovote, P., Krabbe, S., Jacobson, G. A., Müller, C., … Lüthi, A. (2014). Amygdala interneuron subtypes control fear learning through disinhibition. *Nature*, *509*(7501), 453–458. https://doi.org/10.1038/nature13258

Yao, S., Bergan, J., Lanjuin, A., & Dulac, C. (2017). Oxytocin signaling in the medial amygdala is required for sex discrimination of social cues. *eLife*, *6*. https://doi.org/10.7554/eLife.31373

Zahm, D. S. (2006). The evolving theory of basal forebrain functional-anatomical "macrosystems". *Neuroscience and Biobehavioral Reviews*, *30*(2), 148–172. https://doi.org/10.1016/j.neubiorev.2005.06.003

Zancan, M., da Cunha, R. S. R., Schroeder, F., Xavier, L. L., & Rasia-Filho, A. A. (2018). Remodeling of the number and structure of dendritic spines in the medial amygdala: From prepubertal sexual dimorphism to puberty and effect of sexual experience in male rats. *The European Journal of Neuroscience*, *48*(2), 1851–1865. https://doi.org/10.1111/ejn.14052

Zhang, J., Berridge, K. C., Tindell, A. J., Smith, K. S., & Aldridge, J. W. (2009). A neural computational model of incentive salience. *PLoS Computational Biology*, *5*(7), e1000437. https://doi.org/10.1371/journal.pcbi.1000437

Zorrilla, E. P., Logrip, M. L., & Koob, G. F. (2014). Corticotropin releasing factor: a key role in the neurobiology of addiction. *Frontiers in Neuroendocrinology*, *35*(2), 234–244. https://doi.org/10.1016/j.yfrne.2014.01.001

Berridge, Kent C. (2012). From prediction error to incentive salience: mesolimbic computation of reward motivation. The *European Journal of Neuroscience,* 35(7)*,* 1124–1143. doi:10.1111/j.1460-9568.2012.07990.x

Berridge, Kent C. (2019). Affective valence in the brain: modules or modes? Na*ture Reviews. Neuroscience,* 20(*4)*, 225–234. doi:10.1038/s41583-019-0122-8

Berridge, Kent C, & Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *The American Psychologist*, 71*(8)*, 670–679. doi:10.1037/amp0000059

Beyeler, A., Chang, C.-J., Silvestre, *M., Lévêque, C., N*am*bur*i, P., Wildes, C. P., & Tye, K. M. (2018). Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. C*ell reports, 22(*4), 905–918. doi:10.1016/j.celrep.2017.12.097

Bindra, D. (1978). How adaptive behavior is produced: a perceptual-motivational alternative to response reinforcements. *Behavioral and Brain Sciences*, 1*(*01), 41. doi:10.1017/S0140525X00059380

Cai, H., Haubensak, W., Anthony, T. E., & Anderson, D. J. (2014). Central amygdala PKC-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature Neuroscience*, *17*(9), 1240–1248. doi:10.1038/nn.3767

Canteras, N. S., Simerly, R. B., & Swanson, L. W. (1995). Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. *The Journal of Comparative Neurology*, 3*60(*2), 213–245. doi:10.1002/cne.903600203

Castro, D. C., & Berridge, K. C. (2017). Opioid and orexin hedonic hotspots in rat orbitofrontal cortex and insula. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(43), E9125–E9134. doi:10.1073/pnas.1705753114

Chen, P. B., Hu, R. K., Wu, Y. E., Pan, L., Huang, S., Micevych, P. E., & Hong, W. (2019). Sexually dimorphic control of parenting behavior by the medial amygdala. *Cell*, *176*(5), 1206–1221.e18. doi:10.1016/j.cell.2019.01.024

Ciocchi, S., Herry, C., Grenier, F., Wolff, S. B. E., Letzkus, J. J., Vlachos, I., … Lüthi, A. (2010). Encoding of conditioned fear in central amygdala inhibitory circuits. N*ature,* 4*68(*7321), 277–282. doi:10.1038/nature09559

De Boer, S. F., & Koolhaas, J. M. (2003). Defensive burying in rodents: ethology, neurobiology and psychopharmacology. *European Journal of Pharmacology,* 4*63(*1-3), 145–161. doi:10.1016/s0014-2999(03)01278-0

De Olmos, J. S., & Ingram, W. R. (1972). The projection field of the stria terminalis in the rat brain. An experimental study. T*he Journal of Comparative Neurology,* 1*46(*3), 303–334. doi:10.1002/cne.901460303

de Olmos, J. S., & Heimer, L. (1999). The concepts of the ventral striatopallidal system and extended amygdala. A*nnals of the New York Academy of Sciences,* 8*77,* 1–32. doi:10.1111/j.1749-6632.1999.tb09258.x

Deng, H., Xiao, X., & Wang, Z. (2016). Periaqueductal gray neuronal activities underlie different aspects of defensive behaviors. *The Journal of Neuroscience*, *36*(29), 7580–7588. doi:10.1523/JNEUROSCI.4425-15.2016

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science*, *305*(5686), 1014–1017. doi:10.1126/science.1099020

DiFeliceantonio, A. G., & Berridge, K. C. (2012). Which cue to "want"? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. *Behavioural Brain Research*, *230*(2), 399–408. doi:10.1016/j.bbr.2012.02.032

Dong, H. W., Petrovich, G. D., & Swanson, L. W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. B*rain Research. Brain Research Reviews,* 3*8(*1-2), 192–246. doi:10.1016/S0165-0173(01)00079-0

Edmondson, A. J., Brennan, C. A., & House, A. O. (2016). Non-suicidal reasons for self-harm: A systematic review of self-reported accounts. *Journal of Affective Disorders*, 1*91,* 109–117. doi:10.1016/j.jad.2015.11.043

Fadok, J. P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., … Lüthi, A. (2017). A competitive inhibitory circuit for selection of active and passive fear responses. *Nature*, *542*(7639), 96–100. doi:10.1038/nature21047

Fadok, J. P., Markovic, M., Tovote, P., & Lüthi, A. (2018). New perspectives on central amygdala function. *Current Opinion in Neurobiology*, *49*, 141–147. doi:10.1016/j.conb.2018.02.009

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *The Pavlovian journal of biological science*, *15*(4), 177–182. doi:10.1007/BF03001163

Faure, A., Reynolds, S. M., Richard, J. M., & Berridge, K. C. (2008). Mesolimbic dopamine in desire and dread: enabling motivation to be generated by localized glutamate disruptions

in nucleus accumbens. *The Journal of Neuroscience*, *28*(28), 7184–7192. doi:10.1523/JNEUROSCI.4961-07.2008
Faure, A., Richard, J. M., & Berridge, K. C. (2010). Desire and dread from the nucleus accumbens: cortical glutamate and subcortical GABA differentially generate motivation and hedonic impact in the rat. *Plos One*, *5*(6), e11223. doi:10.1371/journal.pone.0011223
Flagel, S. B., Akil, H., & Robinson, T. E. (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. N*europharmacology, 56 Suppl 1,* 139–148. doi:10.1016/j.neuropharm.2008.06.027
Goode, T. D., Ressler, R. L., Acca, G. M., Miles, O. W., & Maren, S. (2019). Bed nucleus of the stria terminalis regulates fear to unpredictable threat signals. *eLife*, *8*. doi:10.7554/eLife.46525
Han, W., Tellez, L. A., Rangel, M. J., Motta, S. C., Zhang, X., Perez, I. O., … de Araujo, I. E. (2017). Integrated control of predatory hunting by the central nucleus of the amygdala. *Cell*, *168*(1-2), 311–324.e18. doi:10.1016/j.cell.2016.12.027
Haubensak, W., Kunwar, P. S., Cai, H., Ciocchi, S., Wall, N. R., Ponnusamy, R., … Anderson, D. J. (2010). Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature*, *468*(7321), 270–276. doi:10.1038/nature09553
Heimer, L, Harlan, R. E., Alheid, G. F., Garcia, M. M., & de Olmos, J. (1997). Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. *Neuroscience*, *76*(4), 957–1006. doi:10.1016/s0306-4522(96)00405-8
Heimer, Lennart, & Van Hoesen, G. W. (2006). The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neuroscience and Biobehavioral Reviews*, *30*(2), 126–147. doi:10.1016/j.neubiorev.2005.06.006
Heyman, G. M., & Dunn, B. (2002). Decision biases and persistent illicit drug use: an experimental study of distributed choice and addiction. *Drug and Alcohol Dependence*, *67*(2), 193–203.
Holland, P. C., & Gallagher, M. (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *The European Journal of Neuroscience*, *17*(8), 1680–1694. doi:10.1046/j.1460-9568.2003.02585.x
Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III--the final common pathway. *Schizophrenia Bulletin*, *35*(3), 549–562. doi:10.1093/schbul/sbp006
Hu, M., & Becker, J. B. (2008). Acquisition of cocaine self-administration in ovariectomized female rats: effect of estradiol dose or chronic estradiol administration. *Drug and Alcohol Dependence*, *94*(1-3), 56–62. doi:10.1016/j.drugalcdep.2007.10.005
Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. doi:10.1038/nature12041
Journal of Comparative Neurology, Volume 35. (1923). Wistar Institute of Anatomy and Biology.
Kikusui, T., Kajita, M., Otsuka, N., Hattori, T., Kumazawa, K., Watarai, A., … Mogi, K. (2018). Sex differences in olfactory-induced neural activation of the amygdala. *Behavioural Brain Research*, *346*, 96–104. doi:10.1016/j.bbr.2017.11.034

Kim, J., Zhang, X., Muralidhar, S., LeBlanc, S. A., & Tonegawa, S. (2017). Basolateral to central amygdala neural circuits for appetitive behaviors. *Neuron*, *93*(6), 1464–1479.e5. doi:10.1016/j.neuron.2017.02.034

Kochli, D. E., Thompson, E. C., Fricke, E. A., Postle, A. F., & Quinn, J. J. (2015). The amygdala is critical for trace, delay, and contextual fear conditioning. *Learning & Memory*, *22*(2), 92–100. doi:10.1101/lm.034918.114

Kravitz, A. V., & Kreitzer, A. C. (2011). Optogenetic manipulation of neural circuitry in vivo. *Current Opinion in Neurobiology*, *21*(3), 433–439. doi:10.1016/j.conb.2011.02.010

Kringelbach, M. L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nature Reviews. Neuroscience*, *6*(9), 691–702. doi:10.1038/nrn1747

LeDoux, J. (2007). The amygdala. *Current Biology*, *17*(20), R868–74. doi:10.1016/j.cub.2007.08.005

Lenoir, M., Augier, E., Vouillac, C., & Ahmed, S. H. (2013). A choice-based screening method for compulsive drug users in rats. *Current Protocols in Neuroscience*, *Chapter 9*, Unit 9.44. doi:10.1002/0471142301.ns0944s64

Lenoir, M., Serre, F., Cantin, L., & Ahmed, S. H. (2007). Intense sweetness surpasses cocaine reward. *Plos One*, *2*(8), e698. doi:10.1371/journal.pone.0000698

Lesscher, H. M. B., & Vanderschuren, L. J. M. J. (2012). Compulsive drug use and its neural substrates. *Reviews in the neurosciences*, *23*(5-6), 731–745. doi:10.1515/revneuro-2012-0066

Li, Y., Mathis, A., Grewe, B. F., Osterhout, J. A., Ahanonu, B., Schnitzer, M. J., … Dulac, C. (2017). Neuronal representation of social information in the medial amygdala of awake behaving mice. *Cell*, *171*(5), 1176–1190.e17. doi:10.1016/j.cell.2017.10.015

Mahler, S. V., & Berridge, K. C. (2009). Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. *The Journal of Neuroscience*, *29*(20), 6500–6513. doi:10.1523/JNEUROSCI.3875-08.2009

Mahler, S. V., & Berridge, K. C. (2012). What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. *Psychopharmacology*, *221*(3), 407–426. doi:10.1007/s00213-011-2588-6

Marcinkiewcz, C. A., Mazzone, C. M., D'Agostino, G., Halladay, L. R., Hardaway, J. A., DiBerto, J. F., … Kash, T. L. (2016). Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. *Nature*, *537*(7618), 97–101. doi:10.1038/nature19318

Miller, S. M., Marcotulli, D., Shen, A., & Zweifel, L. S. (2019). Divergent medial amygdala projections regulate approach-avoidance conflict behavior. *Nature Neuroscience*, *22*(4), 565–575. doi:10.1038/s41593-019-0337-z

Nair, S. G., Gray, S. M., & Ghitza, U. E. (2006). Role of food type in yohimbine- and pellet-priming-induced reinstatement of food seeking. *Physiology & Behavior*, *88*(4-5), 559–566. doi:10.1016/j.physbeh.2006.05.014

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Annals of the New York Academy of Sciences*, *877*, 242–257. doi:10.1111/j.1749-6632.1999.tb09271.x

Nordman, J. C., Ma, X., Gu, Q., Potegal, M., Li, H., Kravitz, A. V., & Li, Z. (2020). Potentiation of Divergent Medial Amygdala Pathways Drives Experience-Dependent Aggression Escalation. *The Journal of Neuroscience*, *40*(25), 4858–4880. doi:10.1523/JNEUROSCI.0370-20.2020

Olney, J. J., Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2018). Current perspectives on incentive salience and applications to clinical disorders. *Current Opinion in Behavioral Sciences*, *22*, 59–69. doi:10.1016/j.cobeha.2018.01.007

Otto, T., Cousens, G., & Rajewski, K. (1997). Odor-guided fear conditioning in rats: 1. Acquisition, retention, and latent inhibition. *Behavioral Neuroscience*, *111*(6), 1257–1264.

Padilla, S. L., Qiu, J., Soden, M. E., Sanz, E., Nestor, C. C., Barker, F. D., … Palmiter, R. D. (2016). Agouti-related peptide neural circuits mediate adaptive behaviors in the starved state. *Nature Neuroscience*, *19*(5), 734–741. doi:10.1038/nn.4274

Paretkar, T., & Dimitrov, E. (2019). Activation of enkephalinergic (Enk) interneurons in the central amygdala (CeA) buffers the behavioral effects of persistent pain. *Neurobiology of Disease*, *124*, 364–372. doi:10.1016/j.nbd.2018.12.005

Paxinos, G., & Watson, C. (2007). The rat brain in stereotaxic coordinates, Ed 6. Amsterdam: Academic.

Peciña, S, & Berridge, K. C. (2000). Opioid site in nucleus accumbens shell mediates eating and hedonic "liking" for food: map based on microinjection Fos plumes. *Brain Research*, *863*(1-2), 71–86. doi:10.1016/s0006-8993(00)02102-8

Peciña, Susana, & Berridge, K. C. (2013). Dopamine or opioid stimulation of nucleus accumbens similarly amplify cue-triggered "wanting" for reward: entire core and medial shell mapped as substrates for PIT enhancement. *The European Journal of Neuroscience*, *37*(9), 1529–1540. doi:10.1111/ejn.12174

Petrovich, G. D., Canteras, N. S., & Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research. Brain Research Reviews*, *38*(1-2), 247–289. doi:10.1016/S0165-0173(01)00080-7

Rasia-Filho, A. A., Fabian, C., Rigoti, K. M., & Achaval, M. (2004). Influence of sex, estrous cycle and motherhood on dendritic spine density in the rat medial amygdala revealed by the Golgi method. *Neuroscience*, *126*(4), 839–847. doi:10.1016/j.neuroscience.2004.04.009

Rasmussen, S., Hawton, K., Philpott-Morgan, S., & O'Connor, R. C. (2016). Why Do Adolescents Self-Harm? *Crisis*, *37*(3), 176–183. doi:10.1027/0227-5910/a000369

Reynolds, S. M., & Berridge, K. C. (2001). Fear and feeding in the nucleus accumbens shell: rostrocaudal segregation of GABA-elicited defensive behavior versus eating behavior. *The Journal of Neuroscience*, *21*(9), 3261–3270.

Richard, J. M., & Berridge, K. C. (2011a). Metabotropic glutamate receptor blockade in nucleus accumbens shell shifts affective valence towards fear and disgust. *The European Journal of Neuroscience*, *33*(4), 736–747. doi:10.1111/j.1460-9568.2010.07553.x

Richard, J. M., & Berridge, K. C. (2011b). Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but D(1) and D(2) together for fear. *The Journal of Neuroscience*, *31*(36), 12866–12879. doi:10.1523/JNEUROSCI.1339-11.2011

Richard, J. M., Plawecki, A. M., & Berridge, K. C. (2013). Nucleus accumbens GABAergic inhibition generates intense eating and fear that resists environmental retuning and needs no local dopamine. *The European Journal of Neuroscience*, *37*(11), 1789–1802. doi:10.1111/ejn.12194

Richardson, N. R., & Roberts, D. C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*, *66*(1), 1–11. doi:10.1016/0165-0270(95)00153-0

Robbins, T. W., Watson, B. A., Gaskin, M., & Ennis, C. (1983). Contrasting interactions of pipradrol, d-amphetamine, cocaine, cocaine analogues, apomorphine and other drugs with conditioned reinforcement. *Psychopharmacology*, *80*(2), 113–119.

Robinson, M. J. F., & Berridge, K. C. (2013). Instant transformation of learned repulsion into motivational "wanting". *Current Biology*, *23*(4), 282–289. doi:10.1016/j.cub.2013.01.016

Robinson, M. J. F., Warlow, S. M., & Berridge, K. C. (2014). Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. *The Journal of Neuroscience*, *34*(50), 16567–16580. doi:10.1523/JNEUROSCI.2013-14.2014

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research. Brain Research Reviews*, *18*(3), 247–291. doi:10.1016/0165-0173(93)90013-P

Sandy, P. T. (2013). Motives for self-harm: views of nurses in a secure unit. *International nursing review*, *60*(3), 358–365. doi:10.1111/inr.12038

Servonnet, A., Hernandez, G., El Hage, C., Rompré, P.-P., & Samaha, A.-N. (2020). Optogenetic activation of the basolateral amygdala promotes both appetitive conditioning and the instrumental pursuit of reward cues. *The Journal of Neuroscience*, *40*(8), 1732–1743. doi:10.1523/JNEUROSCI.2196-19.2020

Steinberg, E. E., Gore, F., Heifets, B. D., Taylor, M. D., Norville, Z. C., Beier, K. T., … Malenka, R. C. (2020). Amygdala-Midbrain Connections Modulate Appetitive and Aversive Learning. *Neuron*, *106*(6), 1026–1043.e9. doi:10.1016/j.neuron.2020.03.016

Stolzenberg, D. S., & Mayer, H. S. (2019). Experience-dependent mechanisms in the regulation of parental care. *Frontiers in Neuroendocrinology*. doi:10.1016/j.yfrne.2019.04.002

Swanson, L W. (2000). Cerebral hemisphere regulation of motivated behavior. *Brain Research*, *886*(1-2), 113–164. doi:10.1016/s0006-8993(00)02905-x

Swanson, L W, & Petrovich, G. D. (1998). What is the amygdala? *Trends in Neurosciences*, *21*(8), 323–331. doi:10.1016/s0166-2236(98)01265-x

Swanson, Larry W. (2005). Anatomy of the soul as reflected in the cerebral hemispheres: neural circuits underlying voluntary control of basic motivated behaviors. *The Journal of Comparative Neurology*, *493*(1), 122–131. doi:10.1002/cne.20733

Takahashi, L. K., Hubbard, D. T., Lee, I., Dar, Y., & Sipes, S. M. (2007). Predator odor-induced conditioned fear involves the basolateral and medial amygdala. *Behavioral Neuroscience*, *121*(1), 100–110. doi:10.1037/0735-7044.121.1.100

Tom, R. L., Ahuja, A., Maniates, H., Freeland, C. M., & Robinson, M. J. F. (2019). Optogenetic activation of the central amygdala generates addiction-like preference for reward. *The European Journal of Neuroscience*, *50*(3), 2086–2100. doi:10.1111/ejn.13967

Tomie, A., Grimes, K. L., & Pohorecky, L. A. (2008). Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. *Brain Research Reviews*, *58*(1), 121–135. doi:10.1016/j.brainresrev.2007.12.003

Torruella-Suárez, M. L., Vandenberg, J. R., Cogan, E. S., Tipton, G. J., Teklezghi, A., Dange, K., … McElligott, Z. A. (2020). Manipulations of Central Amygdala Neurotensin Neurons Alter the Consumption of Ethanol and Sweet Fluids in Mice. *The Journal of Neuroscience*, *40*(3), 632–647. doi:10.1523/JNEUROSCI.1466-19.2019

Treit, D., Pinel, J. P., & Fibiger, H. C. (1981). Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharmacology, Biochemistry, and Behavior*, *15*(4), 619–626. doi:10.1016/0091-3057(81)90219-7

Tye, K. M. (2018). Neural circuit motifs in valence processing. *Neuron*, *100*(2), 436–452. doi:10.1016/j.neuron.2018.10.001

Vanderschuren, L. J. M. J., & Everitt, B. J. (2004). Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science*, *305*(5686), 1017–1019. doi:10.1126/science.1098975

Venniro, M., Russell, T. I., Ramsey, L. A., Richie, C. T., Lesscher, H. M. B., Giovanetti, S. M., … Shaham, Y. (2020). Abstinence-dependent dissociable central amygdala microcircuits control drug craving. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(14), 8126–8134. doi:10.1073/pnas.2001615117

Warlow, S. M., Baumgartner, H. M., Freeland, C. M., Naffziger, E. E., Olney, J. J., Berridge, K. C., & Robinson, M. J. (2020). *The Cambridge Handbook of Substance and Behavioral Addictions*. (S. Sussman, ed.). Cambridge University Press.

Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2020). The central amygdala recruits mesocorticolimbic circuitry for pursuit of reward or pain. *Nature Communications*, *11*(1), 2716. doi:10.1038/s41467-020-16407-1

Warlow, S. M., Robinson, M. J. F., & Berridge, K. C. (2017). Optogenetic central amygdala stimulation intensifies and narrows motivation for cocaine. *The Journal of Neuroscience*, *37*(35), 8330–8348. doi:10.1523/JNEUROSCI.3141-16.2017

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *Journal of comparative and physiological psychology*, *49*(4), 381–391. doi:10.1037/h0088009

Westberry, J. M., & Meredith, M. (2017). Characteristic response to chemosensory signals in gabaergic cells of medial amygdala is not driven by main olfactory input. *Chemical Senses*, *42*(1), 13–24. doi:10.1093/chemse/bjw096

Wilson, T. D., Valdivia, S., Khan, A., Ahn, H.-S., Adke, A. P., Martinez Gonzalez, S., … Carrasquillo, Y. (2019). Dual and opposing functions of the central amygdala in the modulation of pain. *Cell reports*, *29*(2), 332–346.e5. doi:10.1016/j.celrep.2019.09.011

Wolff, S. B. E., Gründemann, J., Tovote, P., Krabbe, S., Jacobson, G. A., Müller, C., … Lüthi, A. (2014). Amygdala interneuron subtypes control fear learning through disinhibition. *Nature*, *509*(7501), 453–458. doi:10.1038/nature13258

Zahm, D. S. (2006). The evolving theory of basal forebrain functional-anatomical "macrosystems". *Neuroscience and Biobehavioral Reviews*, *30*(2), 148–172. doi:10.1016/j.neubiorev.2005.06.003

Zancan, M., da Cunha, R. S. R., Schroeder, F., Xavier, L. L., & Rasia-Filho, A. A. (2018). Remodeling of the number and structure of dendritic spines in the medial amygdala: From prepubertal sexual dimorphism to puberty and effect of sexual experience in male rats. *The European Journal of Neuroscience*, *48*(2), 1851–1865. doi:10.1111/ejn.14052

Zhang, J., Berridge, K. C., Tindell, A. J., Smith, K. S., & Aldridge, J. W. (2009). A neural computational model of incentive salience. *PLoS Computational Biology*, *5*(7), e1000437. doi:10.1371/journal.pcbi.1000437